Annals of Biochemistry and Experimental Medicine

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PREFACE

A three-day symposium on "Amoebiasis and other Intestinal Infections" was held in the Central Drug Research Institute, Lucknow, from the 16th to the 18th November 1959 Contributions had been invited from Indian and foreign laboratories, University institutions and research organizations Over fifty delegates from different research centres in India, USA, Great Britain, France, S Africa and Switzerland participated in the symposium Twentyseven papers on different aspects of the chemotherapy of amoebiasis and three on allied infections were presented

The symposium was inaugurated by Shri V V Giri, Governor of Uttar Pradesh on the 16th November 1959 The Chief Minister of U P, Dr. Sampurnanand, who could not be present due to a previous engagement, sent a message emphasizing the need for such a symposium to evolve methods for the eradication of amoebiasis Professor M S Thacker, in his opening remarks, stressed the need for the development of new and more effective methods of therapy as existing methods of treatment have not proved satisfactory. Lively and illuminating discussions followed almost every paper, the contributions from the assembled scientists were of a high scientific standard and helped to clarify many important points of interest in the problem of amoebiasis

Different sessions were presided over by Col S S Bhatnagar, Hony Professor of Microbiology, St Xavier's College, Bombay, Dr M L Dhar, Assistant Director, CDRI, Lucknow, and Dr K S Sanjivi, Retired Professor of Medicine, Medical College, Madras The symposium concluded with an address by Dr B B Bhatia, Professor of Medicine, K G Medical College, Lucknow I am very much indebted to them for their valuable help and guidance

B Mukerji,
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Proceedings of the Symposium
on Amoebiasis and other Intestinal Infections
held at the Central Drug Research Institute, Lucknow
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INAUGURAL ADDRESS

SHRI V V GIRI
Governor of Uttar Pradesh

I consider it an honour to have been asked to inaugurate this symposium on 'Amoebiasis' this morning and I extend a cordial welcome to the distinguished scientists who have come here to participate in it

Amoebiasis, a type of dysentery, is prevalent all over the world Its incidence is more so in our country both in acute and chronic forms The main causes of this disease are insanitary living conditions, bad water supply, un wholesome and infected food In its chronic type, the disease often recurs making the individual totally invalid and is believed to be incurable

We find references to this malady in our ancient Ayurvedic literature written by Charaka and Sushruta Even to-day we find the age old remedies recommended by them being followed by the people But the last word on a suitable and efficacious remedy is yet to be said and it would be unscientific to accept a cure blind-foldedly simply because we have inherited it from the past It is highly necessary to keep an open mind and do research on these native drugs as much as is being done on modern drugs and antibiotics

While we may continue to take steps to cure those sufferings from this disease, it is equally our bounden duty to formulate measures that would help us in eradicating it totally The foremost thing is to emphasize upon the people the importance of clean living and of following proper hygienic rules

Another reason for the high percentage of the prevalence of this disease is due to the lack of elementary wants to fulfil the needs of the individual for maintaining the health of an individual, such as nutritious food, enough clothing and a little house in healthy surroundings and timely medical attention whenever necessary are to be provided If this is adopted as the basis for our programmes, many of the diseases would fast disappear Efforts should be made to realise the fundamental rights guranteed by the Constitution at the earliest possible opportunity In this task, every section of the community should come forward and co-operate to achieve the cherished goals

The aetiology of this disease was discovered as early as 1875, but still a large number of problems remain to be solved The knowledge we have gathered is only fragmentary, just a handful compared to the ocean of unravelled mysteries A symposium in which eminent experts are participating is bound to contribute much to an understanding of the many issues that confront us That this should

I have much pleasure in inaugurating this symposium and I wish your deliberations every success

MESSAGE FROM CHIEF MINISTER
UTTAR PRADESH

It would have given me much pleasure to be present at this conference. The discussion of the subject is naturally bound to be of a highly technical nature, but I am sure even a layman like me would have found the symposium interesting and instructive. Previous engagements, however, prevent me from accepting the invitation to attend it.

Amoebiasis is a subject of great interest not only to the specialist but to the average citizen as well particularly to residents of tropical countries like India and methods of its eradication deserve the fullest attention of medical men as well as the State. It has been studied from very ancient times. The aetiology, diagnosis and treatment of diarrhoea, dysentery and other diseases of the intestinal region have received very detailed treatment in ancient medical literature which definitely mentions that ailments of this kind may, among other reasons, be due to action of *krimi* (कृमि) that is minute living organisms. The instructions given in these books are based on a long experience of conditions in the climatic and other conditions obtaining in this country and deserve to be seriously studied by medical men even to-day. As is well known, Ayurveda does not stop merely at what might be called the immediate cause namely the amoebae. It tries to go a step further back. It asks the question why a particular patient should be liable to attacks of this kind while others living in the same conditions seem to enjoy a complete immunity? The answer it gives stems from the theory of *Tridosh*. I need not stress this point further.

I have no doubt that the deliberations at this symposium will lead to the enunciation of such practical steps as can be adopted with a view to curb the disease. Curative medicine is useful, of course, but what is most needed is preventive medicine and instruction to the common man in such rules of health as can be easily understood and followed by the average Indian citizen, keeping in mind the social and economic conditions prevailing in the country, specially in our villages.

SAMPURNANAND

OPENING REMARKS

PROF M S THACKER

Secreta y M n s ry of Sc ent fi Resear h and Cultural Affa s and D e tor Gene al Counc l of Sc ent fc and Indust al Re ea h New Delh

(Read by Dr B Mukerj D rector CDRI)

I am happy to have this privilege of associating myself with this symposium and to offer a few remarks as a preliminary to in tiate further and more technical

greater attention and further investigative work in this field of amoebic and

herefore when Dr Mukerji

request to organize an all

and also suggested that the

g international participation

if possible I am glad to see that this objective has been reached to some extent and we have with us some experts from different countries abroad I am con fdent that this jo nt deliberation will be beneficial for all concerned in the develop ment of newer drugs for amelioration and treatment of this type of infection

mosqu to and anti larval measures adopted on a world scale and also the evolu tion of newer and more effective anti malaria remedies Amoebiasis is a disease that has been called a subtle murderer and as such has not received as intensive attent on of world research workers as it deserves It is cosmopolitan in distri bution Specially prevalent in India Indo China Ch na and the Philippines it is common throughout North Central and South Africa Southern United States South America and the West Indies A Russian physician F Losch in St Petersburg in 1875 found amoebae in stools of patients and in ulcers of the colon and was apparently able to transm t the disease to a dog The p oneering work of specially the German workers like Schaudinn and Hartmann and of the British workers like Wenyon and Dobell has clearly shown that several species of amoebae occur in the intestinal canal of human beings Out of these *Entamoeba histolytica* is pathogenic the other species of amoebae are harmless

So little informat on is available regarding the inherent pathogenicity of

amoebic infection Beltran in 1948 pointed out on the bas s of 169 surveys in 43 countries representing more than 350 000 human beings that the global infect on of *E histolytica* is 13 per cent Europe has 10 per cent while the Americans show an average of 12 per cent infection The highest rate was found

for Asia and Africa 16 and 17 per cent respectively In India very little detailed and careful survey has been made so far

The earlier researches gave rise to great controversies concerning the role of *E histolytica* in the causation of amoebiasis Many of these conflicts were eliminated by experimental data from human and animal experiments *E histolytica* can live in the lumen of the large intestine and can invade the mucosa and form ulcers From these ulcers the amoebae frequently move to the liver causing liver abscesses Lung and brain abscesses are rare The later controversies are concerned with the question whether *E histolytica* can live in 'carriers' as a commensal or whether it is always pathogenic or whether the presence of certain types of bacteria is necessary for pathogenicity The demonstration by Boeck and Drbohlav in 1925 of a method of cultivation of *E histolytica* and the discovery of suitable laboratory animals, especially young rats by Jones in 1946 in Britain for the production of experimental amoebiasis are bound to throw light on these important questions

The fragmentary state of knowledge regarding the physiology of *E histolytica* is clearly reflected in the largely empirical approach to the search for effective amoebicides In 1926 Wenyon said that emetine hydrochloride and emetine bismuth iodide were the only drugs considered effective in amoebiasis Since that time a large number of amoebicidal agents including antibiotics have been in current use but most cures that have been followed for considerable periods of post treatment have relapsed As metastatic lesions may also be present in the

chronic course of the disease The ideal chemotherapeutic agent has yet to be discovered

New methods of therapy are needed because of dissatisfaction with the currently available drugs It is to be hoped that the papers and discussions in this symposium may throw light on them I wish your deliberations every success

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CERTAIN ASPECTS OF THE PROBLEM OF AMOEBIASIS

B N SINGH

From the Central Drug Research Institute Lucknow

Amoebiasis is one of the most serious disease caused by protozoa and is

disease to a dog Even after 84 years of the discovery of the aetiology of this disease there are a number of problems in amoebiasis which need solution I shall deal here with only a few of them

The fragmentary state of our knowledge regarding the physiology of amoebae is clearly reflected in the largely empirical approach in the search for drugs to cure amoebiasis Anderson *et al* (3) have listed a total of 27 agents in current use including 4 preparations of emetine 7 aromatic arsenicals 3 iodoquinolines 7 antibiotics 1 kurchi alkaloid 1 German product (Gavano)

and 1 acridine (Rivanol), 2 bismuth compounds and chloroquin Most "cures" that have been followed for considerable periods of post treatment have relapsed This suggests that the antiamoebic drugs discovered, so far, have little or no effect on the cystic stage of *E histolytica* When the effect of the drug disappears, the amoebae come out of the cysts, feed on suitable bacteria present in human intestine, multiply and cause relapse This seems to be the case in chronic amoebiasis for which there is no effective cure It is, therefore, important to discover drugs that should be, in addition to having amoebicidal property, cysticidal or prevent amoebae forming cysts or make the amoebae come out of the cysts This question has not, so far, seriously attracted the attention of biologists and chemists engaged on the chemotherapy of intestinal amoebiasis It seems to me that it is exceedingly difficult, if not impossible, to discover agents that will be cysticidal and will not kill or damage the host Very little, indeed, is known regarding the factor or factors causing encystation in protozoa Moreover, it

that a strain of *Aerobacter aerogenes* produces in an actively proliferating culture, in a synthetic medium, a thermolabile excystment factor More recently, Singh, Mathew and Anand (5) discovered that aqueous extracts of common human intestinal bacteria like *A aerogenes* and *Escherichia coli* cause excystment of viable sterile amoeba cysts A part of the excystment inducing activity was due to the presence of amino acids, some of which have been identified with the aid of paper partition chromatography Some chemically pure amino acids and a few

The factors responsible for the virulence or invasiveness of *E histolytica* are indeed very little understood It has been conclusively established (6) that about 80% of the infections are carrier' cases In such temperate zone as Great Britain the disease does not seem to occur, although 10% of the popula

countries
parts of
riers and
dysentery
or due to

some newly acquired virulent or invasive strain Dew (8) has recently raised the question "Is the concept put forward by Brumpt (9) correct and the amoebae of the temperate zones are a different species, *E dispar*, and that of the tropics,

important in determining whether the large number of contact carrier cases ...
should be treated or not for amoebic infection

The question of host parasite relations in infections with *E histolytica* has been reviewed excellently by Hoare and Neal (10) They have drawn the conclusion "A vast literature has accumulated, dealing with clinical course ... hypotheses have been advanced to explain the

none of these views is sup
admitted that this question
its as experimental animal
parable conditions to man

There is some evidence of the strain differences from acute and contact "carrier" cases It has been demonstrated conclusively by Neal (12, 13), Neal and

Vincent (14) and Singh *et al* (15) that strains from ' carrier ' cases are practically non invasive to rats, though they are able to infect this host Strains from acute [illegible]

differ in virulence but it is not clear whether these differences are innate in the strains Recently, Singh (17) has discovered that two strains of *E histolytica* one isolated in culture from temperate zone (EA strain) and the other from semi tropical zone at Lucknow from 'carrier" cases, become virulent and produce extensive ulceration in the caeca of rats when the amoebae are fed with cholesterol in culture Similarly, these non invasive strains give rise to extensive ulceration when the animals are fed with cholesterol in olive oil, either before or after intra-caecal inoculation of amoebae When the amoebae from extensive ulceration are suspended in normal saline and injected into rats, they retain their virulence through two successive passages in the absence of cholesterol Neal (18) was unable to make EA strain of *E histolytica* virulent by the change in bacterial flora or liver passage in hamsters or by making it encyst in culture Strains from acute cases, that had become avirulent by keeping the amoebae in culture for a long time could be made virulent to rats by hamster liver passage

The unreliability of certain methods used in the laboratory diagnosis of amoebiasis has created much confusion There is much controversy regarding the value of indirect cultural and serological methods The task of detecting

Chaudhuri and Chaudhuri (20) from Calcutta based on their careful work of several years Comparatively low percentage of infection in Lucknow may be due to the climatic factor Lucknow has a dry and hot climate for most of the year and so the survival time of cysts of *E histolytica* outside human body for infecting new hosts may be much less than in humid climates of Calcutta, Madras and Bombay

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DISCOVERY OF DRUGS FOR AMOEBIASIS

F. HAWKING

From the National Institute for Medical Research, London

to make it more active and less toxic, and eventually a compound may be discovered which is sufficiently promising to test in man

The first stage of this process is the "screening test" which is designed to pick out the few active compounds from the large number of inactive compounds

many other infections also The history of the effective compounds which have been discovered in the past emphasizes the need to test a wide range of compounds (because often activity has been discovered in unexpected quarters) and to test compounds on a wide range infections (because compounds synthesized for one purpose have been found active in quite a different manner) The critical evaluation of "active compounds" which may be discovered in this way is left to more elaborate tests

Testing in vitro

Turning now to the specific infection of amoebiasis (due to *Entamoeba histolytica*) in which this Institute is particularly interested, we may say that the simplest and most direct method of testing compounds is to examine their action upon the amoebae *in vitro* The amoebae are suspended in a suitable medium (Balamuth's egg-yolk infusion liver extract) in glass tubes containing suitable concentrations of the new compounds, and they are incubated at 37°C for 24

compounds which are active *in vivo* are inactive *in vitro* and would, therefore, be missed by a purely *in vitro* test Accordingly, most laboratories supplement *in vitro* tests with *in vivo* tests

In vivo *tests*

At the Central Drug Research Institute, tests *in vivo* have been developed in rats Young rats weighing 25-30g are anaesthetized and the caecum is brought to the surface A suspension containing about 50,000-100,000 amoebae in 0 5 cc fluid is injected into the caecum, and the wound is sewn up The rats are treated with the unknown compound by mouth for 5 successive days, starting on the

dose a

rats

rats a

controls, the lesions should be severe (score 4), *i e* the wall of the caecum should be much thickened and ulcerated, the caecal content should consist mostly of mucus without faeces, and many amoebae should be present in the contents and in scrapings from the wall Each drug is given to 4 rats, and the control group contains 6 rats, if the findings in

of activity the compound is tested again

If many compounds are to be tested it

at the first test and to repeat all doubtful results, rather than to use large groups which theoretically might yield more reliable results but which would consume much compound and reduce the number of tests which could be made Most of the compounds submitted to test are inactive and can be discarded after the first trial If a compound shows activity then more detailed tests are required to assess its activity in comparison with that of other compounds Elaborate investigations of toxicity are carried out and eventually (if fortune is extremely favourable) the results may justify a clinical trial

All this work

are many and succ

inspite of disappoinements, *money* to pay for the large expenses involved, and *luck* One often wonders whether *luck* is not the most important ingredient of all

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THERAPEUTIC TRIALS IN AMOEBIASIS

R ELSDON DEW

F om the Amoeb asis Resea ch Un t Durban South Af ca

The Amoeb asis Research Unit* has for many years been engaged in the trial of various ther peutic substances in the treatment of amoebiasis in Africans in Durban In these people for some as yet unknown reason this disease is particularly prevalent some 3000 cases presenting each year at one hospital Liver abscess diffuse peritonitis and other complications are not infrequently seen

*Soonsored by the follow ng bod es The Counc l for Sc ent fc & Industrial Research The Natal Prov nc al Adm n strat on Tl e Un ers ty of Natal and the Un ted States Publ c Health Serv ce (Grant E 1592))

From this plethora of material patients with acute amoebic dysentery of average severity are selected for therapeutic trial Each patient must fulfil the following criteria

1 Actively motile haematophagous amoeba must be found in stools or scrapings from ulcers
2 Ulcers must be visible on sigmoidoscopy
3 There must be neither complications nor co existing disease

In this way, the clinical material is uniform an essential criterion in the comparison of different therapies

Sigmoidoscopy is performed on admission and at frequent intervals there after, usually on the 5th, 10th, 20th and 27th days after the commencement of treatment Stool examination is done at the same intervals and daily if ulcera tion persists after the end of treatment Patients are hospitalised for a minimum of 28 days and are encouraged to return for examination at monthly intervals thereafter At the 5th 10th, 20th and 27th days patients are classified in the following categories

1 Success—symptom free, ulcers healed and no parasites demonstrable
2 Probable failure—persistent ulceration with no parasites demons trable in stools or scrapings
3 Parasitic failure—amoeba still present with or without open ulceration

Once an efficient method of therapy had been established, those patients whose condition was deteriorating and those showing ulceration and trophozoites after the completion of the course of therapy under trial were removed and given the most efficient ther py known

Initially 50 cases were treated by each form of therapy, but, once the more efficient therapies had been established, it was no longer necessary to proceed to 50 cases unless the new drug proved to be competitive The results of some of the trial therapies are given in the Table I which indicates final assessment at 27 days Those drugs or combinations which are not likely to be of interest have been omitted from the list

Discussion

Study of list of trials indicates a process of evolution In the acute form of amoebic colitis, the old stand by emetine, though controlling symptoms, failed to remove parasites in a quarter of the cases, even when given in a dose usually regarded as toxic The higher dose of 15 gr was no more effective than 10 grains Diiodohydroxyquinoline was not significantly better and there was

Carbarsone though showing some activity was not really competitive, and chiniofon only cured some 70% of cases and its side effects were unpopular

It was the investigation of the shot gun' therapy of Hargreaves that opened a new vista When this therapy was split into its components it was realized

TABLE I

Therapeutic trials

Trial no	Therapy	Daily dosage	Days of therapy	No of cases	Assessment at 27 days (%)			Remarks
					Success	Probable failure	Parasitic failure	
1	Emetine	gr i	0 15	50	50	22	28	
2	Di iodohydroxyquinoline	tabs 9	0 20	50	58	18	24	
3	Emetine Di iodohydroxyquinoline	gr i 1 8 g	0 15 0 20	58	64	24	2	
4	Emetine Di iodohydroxyquinoline	gr i 1 8 g	0-10 0 20	62	75	23	2	
7	Emetine Bismuth Iodide Di iodohydroxyquinoline	gr iii 1 8 g	0-10	50	80	14	6	
8	Emetine Carbarsone	gr i gr vii	0 10 0 10	45	58	31	11	
9	Penicillin Succinyl sulphathiazole Emetine *Diiodohydroxyquinoline*	0 3 mega 15 0 g gr i 1 8 g	0 7 0 7 8 22 8 27	59	95	5	0	*Note that* anti amoebic therapy follows anti bacterial
10	Penicillin Succinyl sulphathiazole Emetine Diiodohydroxyquinoline	0 3 mega 15 0 g gr i 1 8 g	0 7 0 7 0-15 0 20	49	90	8	2	Anti amoebic and anti bacterial therapies simultaneous
11	Succinyl sulphathiazole Emetine *Diiodohydroxyquinoline*	15 0 g gr i 1 8 g	0 7 0 10 0 20	49	88	10	2	
12	Penicillin Succinyl sulphathiazole	0 3 mega 1 5 g	0 20 0 20	50	92	2	6	
16	Succinyl sulphathiazole	1 5 g	0-20	57	56	12	34	
17	Penicillin	0 3 mega	0 20	55	46	16	38	
18	Chloroquine diphosphate	1 5 g 1 0 g 0 5 g	0 1 2 5 6 15	10	10	40	50	
20	Carbarsone	gr viii	0 10	50	50	10	40	
21	Emetine bismuth iodide (whole) Phenobarbitone	gr iii *gr i*	0 10 *0 10*	68	44	6	50	Pills appeared in stools
22	Chlortetracycline	1 0 g	0 15	51	94	4	2	

TABLE I (*Contd*)

Trial no	Therapy	Daily dosage	Days of therapy	No of cases	Assessment at 27 days (%) Success	Probable failure	Parasitic failure	Remarks
23	Bismuth glycolylarsanilate	1 5 g	0 10	21	28	10	62	
24	Chiniofon	1 5 g 3 0 g	0 3 4 10	49	71	2	27	
25	Emetine (control)	gr i	0 10	25	56	28	16	
26	Emetine Carbarsone Emetine bismuth iodide Chiniofon	gr i gr viii 3 0 g 1 5 g 3 0 g	0 10 0 10 11 20 11 13 14-20	49	80	18	2	
27	Oxytetracycline	1 0 g	0 15	51	92	2	6	
28	Neomycin	40000 units 40000 units	stat 0 10	12	33		67	
29	Bacitracin	40000 units 40000 units	stat 0 10	11	73	9	18	
30	Neomycin Bacitracin	20000 units 20000 units 20000 units 20000 units	stat 0 10 stat 0-10	12	75	8	17	
31	Emetine bismuth iodide (crushed) Phenobarbitone	gr iii gr i	0 10 0 10	53	70	9	21	
32	Entamide	5 g	0 10	10	30	30	40	
33	Entamide benzoate	5 g	0 10	10	40	30	30	
34	Streptomycin	2 0 g	0 10	17	12	—	88	29% cyst passers after therapy
35	Chloramphenicol	1 0 g	0 15	5	40	—	60	
36	Chloramphenicol (enteric coated)	1 0 g	0 15	8	38	—	62	
37	Chloramphenicol	4 0 g 2 0 g	0-3 4-12	16	62	—	38	
38	Polymyxin	0 4 g	0 10	7	14	—	86	
40	Simaroubidin	50 mg	0 10	8	38	—	62	
41	Simaroubidin	0 2 g	0-10	49	47	—	33	

TABLE I (Contd)

Trial no	Therapy	Daily dosage	Days of therapy	No of cases	Assessment at 27 days (%)			Remarks
					Success	Probable failure	Parasitic failure	
42	Fumagillin	40 mg	0-10	7	—	—	100	
43	Fumagillin	200 mg	0-10	47	70	4	26	Toxic symptoms
44	Fumidil	60 mg	0-10	11	18	—	82	
45	Emetine bismuth iodide (enteric)	gr iii	0-10	41	80	5	15	
48	Food only	—	—	10	—	—	100	
51	Penicillin Diiodohydroxyquinoline Emetine	0.2 mega 1.8 g gr i	0-10 0-20 0-10	53	79	11	10	
53	Oxytetracycline residue	4.0 g	0-10	9	67	—	33	1g 11.0 mg oxytetracycline
54	Oxytetracycline intra muscular	0.2 g	0-10	5	40	20	40	
55	Chlortetracycline	80 mg	0-10	10	60	—	40	
56	Streptomycin residue	4.0 g	0-10	10	10	10	80	30% cyst passers after therapy
57	Chlortetracycline residue	4.0 g	0-10	13	77	—	23	1g 20.0 mg chlortetracycline 15% cyst passers
59	Tetracycline	1.0 g	0-10	59	97	—	3	
60	Puromycin	0.5 g	0-5	10	40	—	60	
61	Diphetarsone	2.5 g	0-10	43	74	7	19	
62	Entamide (enteric coated)	5.0 g	0-10	10	—	40	60	
63	Erythromycin	2.0 g	0-10	21	71	14	10	One case (5%) showed toxicity
64	Chlortetracycline Diiodohydroxyquinoline Chloroquine	100 mg 1.8 g 0.5 g	0-10 0-20 0-15	48	94	2	4	
65	Spiramycin	2.0 g	0-10	30	83	7	6	One case (4%) developed liver abscess
66	Novobiocin	1.0 g	0-10	10	30	20	50	
67	Novobiocin	2.0 g	0-10	9	33	11	56	
69	Aminitrazole	300 mg	0-10	13	31	8	61	

and parasitic cure indicated the importance of the part played by the bacteria associated in the genesis and maintenance of the disease

This led to an antibiotic approach, and the tetracyclines gave a success rate unprecedented by any other single drug therapy Apparently, the wider the anti bacterial spectrum the more effective the therapy, whether this was achieved by a single drug or by a combination Seemingly, the varied nature of the bacterial species sustaining the amoeba implies the use of a therapy likely to hit as many different species as possible

A series of antibiotics were tried, with some surprising results Chloramphenicol, for example converted a high proportion of cases into cyst passers, leading to some speculation as to the role of the surviving bacterial species in the maintenance of the commensal state of the amoebae

Antibiotics aimed directly at the amoeba were tried Fumagillin which *in vitro* had shown extreme promise, failed in the human, and was relatively ineffective even in toxic doses

The high cost and the dangers of the use of massive doses of tetracycline led to the use of smaller doses and though these were not as dramatic as the more drastic therapy they were effective However the development during anti bacterial therapy of such complications as liver abscess, and the continued occurrence of some relapses indicated that this approach is not the complete answer It is apparent that in our present state of knowledge the therapeutic attack must be aimed at (*i*) the amoeba in the lumen, (*ii*) the amoeba devouring the bowel wall and (*iii*) the amoeba in the tissues The obvious drugs are a

he most
effective
ever, to

of the tetracyclin
this dosage the d
to potentiate the
to use somewhat higher dosages of tetracycline

However, our results so far merely indicate the necessity for continued study, possibly in the direction of some anti metabolite to divert that essential substance which the amoeba obtains from bacteria in the bowel and from man in the tissues

Trials continue

Summary

A review is given of the therapeutic trials in acute ulcerative amoebic colitis, carried out by the Amoebiasis Research Unit in Durban, South Africa Over eighty forms of therapy have been tried

Acknowledgment

The extensive clinical work was carried out by Dr F G Armstrong, Dr R J Marot, Dr A J Wilmot, Dr N R Pooler and Dr S J Powell

These trials would not have been possible without the co operation of the Director of the Hospital Services Dr J Parker, and of the Superintendent of King Edward VIII Hospital, Dr S Disler

I wish to thank the various drug companies for the supply of many of the materials under test

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EXPERIMENTAL CHEMOTHERAPY OF AMOEBIASIS

R N CHAUDHURI T K SAHA AND N ROY

F om I e Cal tta S hool of Trop al Med ne Calcutta

Drugs showing anti amoebic properties *in vitro* are usually evaluated in experimental animals before subjecting them to clinical trial In the past the testing methods utilized experimental infections in kittens dogs or monkeys or natural amoebiasis in monkeys Owing to practical difficulties smaller mammals are now commonly used for the purpose Of the rabbits guineap gs hamsters

made between the lumen of the caecum and the exterior either on the ventral (Fig 2) or on the dorsal aspect (Fig 3) of the animals A set of washer and a nut fixes the tube to the parietal wall and a cap at the outer end prevents leakage of caecal contents About the tenth day of operation the an mal is ready for experiment it lives normally with the tube *in situ* for an indefinite period Utili zing this technique some observations have been made firstly on the result of inoculation through the tube and secondly on the effect of amoebicidal drugs also introduced through the tube The aim and object had been to see if the

cl nical relapse

per i
daily
and
numbers indicat ng *onset* of infection which occurred usually between the 5th and 12th day of inoculation More than half of the animals became infected

Lower part of the colon also

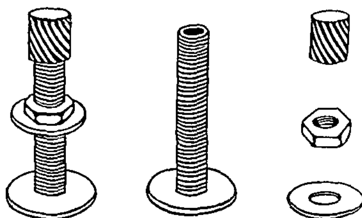


Fig 1 Fixture with its different parts



Fig 2 Guineapig with ventral fixation



Fig 3 Guinea pig with dorsal fixation

In another series of animals an amoebicidal drug was administered at about the onset of infection and the progress was assessed by daily examination of caecal contents for amoebae Seven drugs were arbitrarily used in 63 infected animals as indicated below by the following preliminary trials

1 *Emetine bismuth iodide*—It was given to 7 infected guineapigs in dosage of 8 mg once daily for 10 days In 3 of them the caecal content became negative on the 4th 6th and 7th day of treatment one however died 2 days after the course The remaining 4 animals died between the 5th and 10th days of treatment with the persistence of infection In the 2 animals apparently responding to therapy the caecal content was positive 9 and 16 days after completion of treatment They also died soon after

2 *Carbarsone*—It was given in daily dosage of 25 mg to 4 animals The

weeks

Another 4 infected animals were given a smaller dosage of 15 mg daily for 10 days Three failed to respond and died between the 6th and 10th days of treatment In the fourth the caecal content became negative on the 4th day, the animal remained well for 4 weeks after completion of the course Thereafter, *E histolytica* reappeared in the caecal content

3 *Kurchi bismuth iodide* (prepared in the Chemistry Department) was tried in 12 infected guineapigs the dose being 60 mg once daily for 10 days In 7

the amoebae disappeared from the caecal content between the 2nd and 7th days of treatment, but reappeared between 15 and 48 days after the treatment In the remaining 5 they persisted throughout the period

4 *Ipecac bismuth iodide* (prepared in the Chemistry Department) was given to 12 animals in dosage of 6 mg once daily for 10 days In 4 of them, the caecal content was negative on the 3rd, 6th, and 8th day of treatment respectively Two of them died on the 7th and 8th days with liquid motions, although the caecal content was free from amoebae and the other 2 became positive again on the 27th and 35th days after completion of therapy All the other 8 animals died between the 5th and 10th days of therapy with persistence of amoebae in the caecal content Five of them also had loose motions with frank liquid character of caecal contents

5 *Mebinol V* (Carlo Erba) —This drug (ethoxy-ethyl phenoxy nitrobenzyl

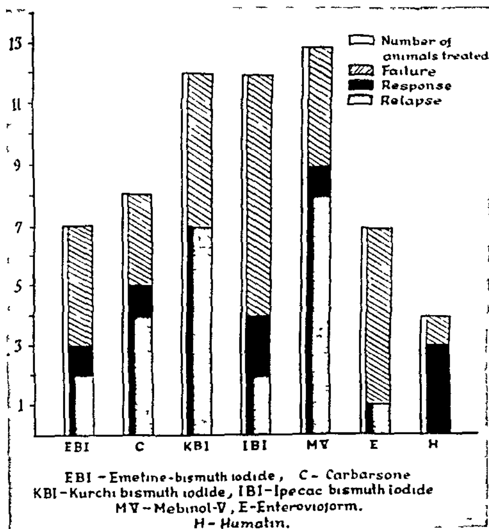


Fig 4 Graph showing results of screening amoebicidal drugs

[illegible]

amoebae in the caecal content

6 *Enterovioform* (CIBA) was given to 7 infected guineapigs in a dosage of 125 mg once daily In 6 of them it had no effect as was evident from the persistence of amoebae in the caecal contents and all of them died within 5 and 11 days One became negative on the 5th day but was positive again 11 days after the course.

7. *Humatin* (*Paromomycin*) —This antibiotic obtainable from cultures of [illegible] was tried in 4 infected

[illegible]

The remaining animal died on the 5th day All the 3 animals responding to the treatment remained well with negative reports throughout the period of observation for a month

The accompanying graph (Fig 4) represents the preliminary chemotherapeutic response *in vivo* to the seven drugs in the doses mentioned

Without attempting to draw any conclusion on the relative efficacy and toxicity of the particular drugs from the above *in vivo* study, it appears that the experimental device evolved promises to be of value not only in observing the natural course of *E histolytica* infection in guineapigs but also in assessing the effect of different compounds on amoebae in the colon The animals that would respond and survive the infection with disappearance of parasites from the caecal content may also be followed up for possible recurrence, chronicity and other studies The work is in progress in the clinical Research Unit, Indian Council of Medical Research

Our thanks are due to Shri P Ghose for technical assistance

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AN APPROACH TO THE CHEMOTHERAPY OF AMOEBIASIS

M L DHAR

From the Central Drug Research Institute Lucknow

Dr B Mukerji gave a review of important landmarks in the chemotherapy of amoebiasis yesterday and there is one scheduled from Dr J Druey for this session of the s[illegible] I shall restrict m[illegible] remarks to the [illegible]

way open to the workers in this important field It is, however, probably possible to introduce a degree of rationale in this empiricism In our laboratories, we felt that emetine and conessine, the two powerful systemically active amoebicides, may provide leads to an attack on this problem

In an assessment of the possible mode of drug receptor association, we concluded that hydrogen bonds aided by van der Waals' associations were operative in the case of emetine and conessine I discussed this subject in detail in an

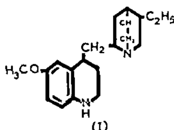
protein helix

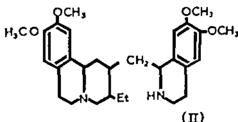
The simple diamines bearing an electronic status and the intervening distance of the two nitrogen atoms nearly equivalent to those of emetine and conessine were shown to possess high *in vitro* activity (2) Major deviation from these conditions led to the abolition of activity Likewise, restriction of the mobility

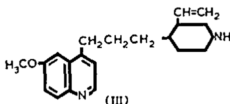
activity was dependent also on the steric disposition of the related molecular

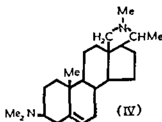
molecules by these forces
without the aid of electrost
the interacting molecules in

tive agent It is
between desoxy
r ethyl, piperidyl
he rupture of the









9 Chloro 9 desoxyquinine, obtained by the treatment of quinine monohydrochloride with thionyl chloride or phosphorus pentachloride, gave on reductive dehalogenation with hydrogen in presence of palladium charcoal, 9 desoxydihydroquinine which was reduced further by the method of Pyman for the reduc
For obtain
quinine was
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orresponding

experimental

bioreceptor

quinolines (15) These latter compounds were prepared with a view to provid ing alternate centres for chelation in the quinoline molecule and to find out if they retained antiamoebic activity Since 8 hydroxyquinolines are held by the
ole chemo-
4, 5-, 6
tive both

e g 4 b-

Others are still under test

I may suggest that the activity of the 8 methoxy quinolines quinaldines quinazolines and quinozolones would perhaps not be considered to be dependent on chelation unless demethylation preceeds actively in the biophase, and that these may act by a different mechanism possibly one that governs the activity of *chloroquin*

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14 [illegible]

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IN VITRO APPROACHES TO CHEMOTHERAPY OF AMOEBIASIS

P S KAUSHIVA

From the Central Drug Research Institute, Lucknow

determining whether or not a compound has any specific anti amoebic effect

METHODS

The chief drawback in conducting *in vitro* tests is that so far it has not been possible to cultivate *E histolytica* in the test tube free from other living cells and this fact poses several problems On a closer examination, it will be worth-

(1) *Test medium* The choice of test medium in a drug testing programme is of great importance A prerequisite is that the medium should be able to support the growth of amoebae as well as the concomitant associates Numerous media exist but the simplest possible media are preferable in order to minimise the side-effects of the ingredients Egg extracts (egg eluates 1 2) provide a simple medium for conducting drug tests and all fluid (monophasic) media is generally employed in comparison to biphasic media because of ease in handling and to ensure uniform distribution of drugs The presence of special substances (free proteins like serum (3) —SH group (4 5) and pH in the medium (6,7) influences the drug activity Reardon and Rees (8) have done the evaluation of drugs upon *E Histolytica* without overlay serum The present trends have also led to the development of all fluid essentially synthetic medium of

epted that intestinal amoebae require
is requirement is either met by special
l) or the reducing conditions are pro-
duced by the physicochemical conditions created by the microbial associates

distinguish between direct and indirect activity of chemical agents Duration of incubation is also an important factor in antiamoebic action and sufficient attention should be paid to the period of drug exposure The *in vitro* sensitivities

TABLE I

In vitro *screening methods employed in various laboratories*

No	Test medium compositions	Incubation duration (hr)	Inoculum: Associates	Inoculum: Amount (per cc)	Criteria of endpoint: Direct examination & subcultivation	Criteria of endpoint: Associates
1	Serum Ringer (preconditioned)	72	Monobacterial		Direct examination	Methylene blue
2	Serum Ringer	24	*Polybacterial*		Direct examination & sub-cultivation	—
3	Liver egg yolk	48	(a) Monobacterial (b) Polybacterial	3×10^4	Direction examination sub cultivation	(a) O/R potential (b) Population
4	Egg eluate	(a) 24	Polybacterial	3×10^4	Direct examination	—
		(b) 24 48	Polybacterial	5×10^4	Direct examination & sub cultivation	—
		(c) 24 48	Polybacterial	Massive	Direct examination	Use of drug fast bacteria
5	Liver protease peptone	48	Monobacterial	3×10^4	Direct examination & sub cultivation	(a) O/R potential (b) Respiration
6	Trypticase-thio glycolate blood (preconditioned)	72	*T cruzi*	1	Direct examination	motility
7	Egg slant Locke (biphasic	(a) 48	*T cruzi*		Direct examination & sub cultivation	Population
		(b) 48	*T cruzi*		Direct examination & sub-cultivation	Population
		(c) 48	Monobacterial	500	Direct examination & sub cultivation	Population
8	Serum liver extract (micromethod)	(a) 24	Polybacterial			—
		(b) 48	Polybacterial	3×10^4	Direct examination	—
		(c) 24-48 (horizontal)	Polybacterial	10 amoebae /1/6 high power	Direct examination	—

of four strains of *E histolytica* have been studied by de Carneri (11) after various periods of incubation The effect of drug incubation period also depends upon the nature of test substance Some highly effective direct acting amoebicides (*cf* actidione) have much less effect at 24 hr than at 48 hr However, a longer period of incubation entails the risk of confusing antibacterial activity with antiamoebic activity This risk is worth taking in the case of synthetic compounds In our experience most of the synthetic compounds tested in the laboratory showed hardly any difference in dilution endpoints at 24 and 48 hr intervals

TABLE I (*lateral continuation*)

No	Reference	Amoebicidal endpoints of some selected drugs (μg/cc)			
		Emetine	Vioform	Carbarsone	Newer compounds
1	Dobell (14)	5	—	—	—
	Goodwin (24)	10	—	100	—
	Fulton (25)	5	—	—	—
	Neal (26)	5	—	—	10 (Mantomide and Entamide
	Kradolfer (27)	[illegible]0	2500	1200	60 (Entobex)
2	Kaushiva (16 19)	8	—	—	—
3	Balamuth (10 22	5	100	—	0 01 (Fumagillin)
4	(a) Thompson (28)	4 5	15	1000	
	(b) Kaushiva (29 30)	3 9	10	—	—
	(c) Thompson (1, 2	10	—	—	3 9 (Humatin)
5	Anderson (13)	10	100	2000	—
6	Phillips (12)	5	—	—	—
7	(a) Anderson (13)	—	—	—	0 1 (Fumagillin)
	(b) Bradner (31)	31 3	—	—	—
	(c) Brackett (32)	200	80	—	—
8	(a) Rawson (33)	62 5	—	—	—
	(b) Lynch (34)	3 9	—	—	0 03 (Fumagillin) 12 5 (Mantomide)
	(c) Woolfe (21)	2	1 100	—	0 01 (Fumagillin) 1 0 (Mantomide) 0 1 (Entamide)

(iii) *Inoculum* Both the quantitative (amount, age, growth phase) and qualitative (strain and species differences, culture associates) aspects of the inoculum are important The inocula have varied quantitatively from undeter-

microbial associates Generally, no safeguards

are taken against confusing bactericidal action with amoebicidal action As already stated the presence of associates complicates the evaluation of amoebicidal drugs particularly in the assessment of antibiotics Balamuth (10) used large inoculum and recorded pH and viable bacterial populations at the apparent endpoints coupled with subcultivation of all negative tubes to eliminate cases of inhibition

The associates have varied from unknown mixed bacterial flora (polybacterial) to single species of bacteria (monobacterial) and in some cases have been the

fact that it is more resistant than bacteria to most antibiotics and other amoebicidal drugs The methods employed for assessing the susceptibility of the associates include the study of populations and effect on motility Biochemical indices have been measured in terms of reducing activity of the medium (O R potential) (10) reduction of indicator dyes (14) and oxygen consumption of the associates (13)

Ultimately, drugs under test are evaluated in terms of amoebicidal activity and it is highly desirable to standardize the following parameters in testing (a) numbers of amoebae in inoculum (b) criterion of amoebicidal endpoint for complete kill (c) probable effect of agents upon the bacterial flora It is necessary to employ subcultivation into preconditioned normal culture medium and to preferably employ monobacterial cultures Amoebicidal endpoints are

involved in the tests and lacl
information on the range of
of sub lethal concentration c
in cultures have been analy
of surviving amoebae can be determined more accurately and the errors in ascertaining death of a culture are eliminated The extent and nature of damage can also be assessed by exposing them to sub lethal concentrations and examining

The choice of amoebae other than the causative agent as test objects for rapid and large scale screening of amoebicidal compounds depends on the assumption that if the sensitivities of *E histolytica* against different amoebicides could be

and *Didascalus thorntoni*) and *E histolytica* has been made (16) A similar comparative study on the response of *E invadens* and *E histolytica* to several drugs has been made by McConnachie (17) and de Carneri (18) and they have discussed the significance of their studies on the use of *E invadens* for chemotherapeutic research in amoebiasis This may prove fruitful by drawing a parallel with the approaches leading to the development of the antimalarial Daraprim'

Laboratory Studies

various investhe following and *Holarrhena* es, (*iv*) arsonic ide of place being amoebicidal at low concentrations and for its direct action as it is not antibacterial even at high concentrations, for these reasons it has served as a standard by which the effect of other drugs is measured. Emetine also shows differential action against *E histolytica* in comparison to other species of intestinal amoebae (10) In Table II, the dilution endpoint activity of emetine and conessine

TABLE II

Sensitivities of amoebae to emetine and conessine

Experiment No	Emetine (µg /cc) *E histolytica*	*S russelli*	*D thorntoni*	*N gruberi*	Conessine (µg /cc) *E histolytica*	*S russelli*	*D thorntoni*	*N gruberi*
1	8	500	250	63	63	8	16	31
2	8	125	125	31	31	8	4	31
3	4	250	125	250	63	8	4	31
4	8	125	125	31	63	63	4	4
5	8	31	125	63	31	16	125	4
6	2	500	125	500	31	16	31	4
7	8	31	63	250	63	4	4	31
8	4	125	63	31	31	8	4	31
9	8	125	125	63	63	8	8	4
10	8	125	125	63	63	8	4	31
Likely amoebicidal endpoint	8	125	125	31	63	8	4	31
Mean amoebicidal endpoint	6	194	125	135	50	15	10	20

against different species of amoebae are given The noteworthy difference is

activity is strangely limited to species belonging to the genus *Entamoebae* and appears to be inactive *in vitro* against *Hartmannella castellani*

A comparison of amoebicidal activity of a number of compounds against different species of amoebae is given in Table III The susceptibilities of amoebae

TABLE III

Amoebicidal end points (μg /cc) of arsenic derivatives of thiazole and thiazolidone and some polymethylene diamines

Series	Compd no	R	R	E histolytica	S russelli	D thorntoni	N gruberi
R—C—N / HC C—NH CO R / S							
(M)	M1	C_6H_5	C_6H_4AsO	2	2	4	63
	M2	C_6H_4Cl (*p*)	—	250	500	125	500
	M3	$C_{10}H_7(\alpha)$	—	8	16	63	16
(N)	N1	C_6H_5	$C_6H_4AsO_3H_2$	31	31	16	17
	N2	C_6H_4Cl (*p*)	—	250	250	250	500
	N3	$C_{10}H_7$ (α)	—	125	125	125	125
	N4	$C_6H_4CH_3$ (*p*)	—	4	8	8	8
	N5	$C_6H_4OCH_3$ (*p*)	—	>1000	>1000	>1000	>1000
OC—NH / H₂C C=NR CO R / S							
(Q)	Q1	$C_6H_4CH_3$ (*p*)	C_6H_4AsO	16	500	1000	1000
	Q2	C_6H_4Cl(*m*)	—	4	250	125	63
	Q3	C_6H_4Cl(*p*)	—	31	250	125	16
	Q4	$C_{10}H_6(\alpha)$	—	125	250	500	125
(P)	P1	C_6H_4	$—CH_2NHC_6H_4AsO_3H_2$	125	1000	500	1000
	P3	$C_6H_3CH_3$ (*p*)	—	125	250	500	500
	P4	C_6H_3Cl(*p*)	—	250	500	500	500
	P7	$C_6H_3NO_2$ (*p*)	—	250	>1000	>1000	>1000
	P9	C_6H_3COOH (*m*)	—	16	>1000	1000	>1000
	P10	C_6H_3COOH (*p*)	—	31	1000	250	500
	P11	$C_{10}H_6$ (α)	—	250	1000	>1000	500
	P12	$C_{10}H_6$ (β)	—	1000	500	500	250

TABLE III (Contd)

Amoebicidal end points (μg/cc) of arsenic derivatives of thiazole and thiazolidone and some polymethylene diamines

Series	Compd no	R	R	*E histolytica*	*S russelli*	*D thorntoni*	*N gruberi*
$H_2O_3AsH_4C_6$—CH(—S—)C—NR, ring closed by CO—NH							
(R)	R1	C_6H_5		250	1000	1000	125
	R2	$C_6H_4CH_3$ (o)		1000	1000	1000	250
	R3	$C_6H_4CH_3$ (p)		500	500	250	125
	R4	C_6H_4Cl (p)		125	500	1000	1000
	R8	C_6H_4COOH (o)		1000	500	500	>1000
	R9	C_6H_4COOH (m)		>1000	1000	>1000	>1000
	R10	C_6H_4COOH (p)		>1000	1000	1000	>1000
	R11	$C_{10}H_7$ (α)		250	500	500	250
$(n\text{-Bu})_2N(CH_2)_3NHR$							
(A)	69	n C_6H_{13}		16	31	31	31
	70	n C_7H_{15}		16	31	16	31
	71	n C_8H_{17}		1	16	31	31
	72	n-$C_{12}H_{25}$		1	2	4	8
	73	—$C_6H_4CH_3$(p)		16	8	8	4
	76	Benzyl		31	16	31	31
	77	$(CH_2)_2Ph$		4	16	31	8
(piperidino) —$(CH_2)_3NHR$	78	n C_3H_7		63	250	31	1000
	79	n C_4H_9		63	500	250	1000
	80	iso-C_4H_9		63	1000	250	500
	83	n C_6H_5		4	250	63	63
	84	n-C_7H_{15}		4	250	31	63
(B)	85	iso-C_8H_{17}		2	31	4	8
	86	n $C_{12}H_{25}$		2	8	4	2

TABLE III (*Contd*)

Amoebicidal end points (μg /cc) of arsenic derivatives of thiazole and thiazolidone and some polymethylene diamines

Series	Compd no	R	R′	*E histolytica*	*S russelli*	*D thorntoni*	*N gruberi*
—$(CH_2)_3NHR$ (C)	87	Ph		16	16	31	16
	88	—$C_6H_4OCH_3$ (*o*)		16	4	8	16
	89	—$C_6H_4OCH_3$ (*p*)		31	63	31	63
	90	Benzyl		8	8	16	8
	91	$(CH_2)_2Ph$		8	250	125	250
	100	*n* $C_{11}H_{23}$		4	8	8	2
	102	—$C_6H_4OCH_3$ (*o*)		16	16	16	8
	104	Benzyl		125	63	63	63
$R(CH_2)_6R$ (D)	106	(C_2H_5)-NH—		8	1000	1000	1000
	107	(*n* C_4H_9)-NH—		16	500	125	16
	108	(*n* C_6H_{13}) NH—		8	63	16	63
	109	(*n* C_7H_{15}) NH—		16	250	31	250
	110			63	1000	1000	1000
	111			31	1000	500	500

[illegible]

E histolytica and 3 species of free-living amoebae against certain amines and arsenic derivatives of thiazolidone and thiazole show that free-living amoebae among themselves show a somewhat parallel behaviour in their response to the various compounds but their amoebicidal endpoints are generally different as compared to *E histolytica* However, correlation in the activity of some series of [illegible] For instance, [illegible] various amoebae [illegible] ne exist in the thiazolidone compounds (Table IV) It could be, perhaps, that thiazole ring is the molecular component active against all the amoebae. Among the polymethylene diamines, the morpholino and piperidino substituted nonanes show a significant level of correlation while butyl and disubstituted nonanes show no correlation

TABLE IV

Correlation between sensitivities of Entamoeba histolytica *and free living amoebae*

Series	*S russelli*	*D thorntoni*	*N gruberi*
M	0 9999*	0 8849	0 9941
N	0 9923*	0 9998*	0 9638*
Q	—0 3421	0 3575	—0 2694
P	0 4619	0 1395	0 4014
R	—0 1059	—0 1067	—0 5713
A	0 3250	0 1952	0 3593
B	0 6840*	0 7135†	0 8803†
C	0 9990*	0 9990*	0 9999*
D	0 5513	0 5325	0 5226

*Significant at 5% level
†Significant at 1% level

In vitro methods bring out elegantly the relation between chemical structure and amoebicidal activity and provide suitable leads to the chemist for future synthesis of compounds besides eliminating less active compounds in an early stage of a screening programme However, every testing procedure has its limitations and *in vitro* methods are not considered adequate in the case of antibiotics and insoluble compounds Some of the insoluble benzacridine derivatives tested in our laboratory showed no activity in *in vitro* tests but were mildly active in *in vivo* tests against rats (Table V) In such cases, endpoints arrived at are not unequivocal and should be scrutinised carefully Another disadvantage inherent in these tests is that sometimes it is not possible to differentiate between specific amoebicidal action and general protoplasmic toxicity

CONCLUSIONS

In spite of some complexities and drawbacks *in vitro* methods have a definite role to play in the critical evaluation of amoebicidal drugs They are direct and simple requiring small amount of test compounds Also, they are relatively less expensive and are rapidly completed The use of *in vitro* techniques has often

TABLE V

Amoebicidal activity of some benzacridine derivatives

Compd no	Nomenclature	*In vitro* minimal amoebicidal conc (μg/cc)	Oral dose (mg/kg) daily/6 days	*In vitro* activity*				Remarks
				Average score amoebae		Appearance		
				Scraping of wall	Contents	Wall	Contents	
AFMC 1	7 *p*-Chloroanilinobenz/C/acridine HCl	100	300	2 5	0 7	3	3	Probably inactive
AFMC 2	7-*p*-Toluidinobenz/C/acridine HCl	100	300	1 7	1 7	1 7	0 7	Moderately active
AFMC 3	7 *p* Bromoanilinobenz/C/acridine HCl	100	300	2	2	1 7	1 2	Moderately active
AFMC 4	12 *p*-Iodoanilinobenz/A/acridine salicylate	100	300	3	3	2 6	3 2	Slightly active
AFMC 5	12-*m* Iodoanilinobenz/A/acridine salicylate	100	300	1	1	1	0 5	Moderately active
AFMC 6	12-*p*-Anisidinobenz/A/acridine salicylate	10-100	300	1 2	1 2	2	1 5	Slightly active
				Control	4 4	3 6	4	

* Four rats were used in controls and 4 rats for each compound

TABLE VI

Amoebicidal activity of some phenylamines

SERIES	COMPOUND NO.	SUBSTITUENTS	IN VITRO MINIMUM AMOEBICIDAL CONCENTRATION (μg/ml)
(A) CH_3O, CH_3O–phenyl–R, –CH–NH_2 HCl, CH_3	BP(C) 4	$-CH_3$	> 1000
	BP(C) 5	$-C_2H_5$	500
	BP(C) 6	$-C_3H_7$	500
	BP(C) 7	$-C_4H_9$	125
	BP(C) 2	$-C_6H_{13}$	4
(B) CH_3O, CH_3O–phenyl–R, –CH_2–CH_2–NH_2.HCl	BP(C) 8	$-CH_3$	250
	BP(C) 9	$-C_2H_5$	250
	BP(C) 10	$-C_3H_7$	250
	BP(C) 11	$-C_4H_9$	125
	BP(C) 1	$-C_6H_{13}$	4
(C) CH_3O, CH_3O–phenyl–CH–R, –NH_2 HCl	BP(C) 3	$-C_5H_{11}$	8—16
(D) CH_3O, CH_3O–phenyl–CH–R–, –NH $(CH_2)_2$–NH_2 HCl	CNK 1	$-C_2H_5$	125
	CNK 2	$-C_3H_7$	63
	CNK 3	$-C_4H_9$	31—63
	CNK 4	$-C_5H_{11}$	16
(E) CH_3O, CH_3O–phenyl–R, $(CH_2)_n$ NH_2.HCl, n = 3	CNK 5	$-CH_3$	16
	CNK 6	$-C_2H_5$	16
	CNK 7	$-C_3H_7$	16
(F) n = 4	CNK 8	$-C_3H_7$	8
	CNK 9	$-C_4H_9$	4—8
(G) CH_3O–phenyl(R)–CH CH_3, NH_2 HCl	SC NK 1	$-CH_3$	> 100
	SC NK 2	$-C_2H_5$	> 100
	SC NK 3	C_3H_7	> 100
	SC NK 4	$-C_4H_9$	100
	SC NK 5	C_5H_{11}	> 100
	SC NK 6	C_6H_{13}	10 100
(H) CH_3O–phenyl(R)–$(CH_2)_2$ NH_2 HCl, n = 2	SC NK 7	CH_3	> 100
	SC NK 8	$-C_3H_7$	100
(I) n = 3	SC NK 9	CH_3	> 100
	SC NK 10	C_2H_5	100
	SC NK 11	$-C_3H_7$	100
(J) n = 4	SC NK 12	CH_3	> 100
	SC NK 13	$-C_2H_5$	> 100
	SC NK 14	$-C_3H_7$	10-100
	SC NK 15	$-C_4H_9$	10

been questioned on the ground that drugs which undergo *in vivo* transformations would be missed as would also those drugs which have no activity *per se* but which help the body to overcome the infection Such drugs are very rare and the risk involved is justifiable (21) It is to be remembered that there is no amoebicidal drug available which does not possess marked antiamoebic effect demonstrable by tests done outside the body system

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AMOEBIASIS—A CLINICAL RETROSPECT

K S SANJIVI*

The incidence of amoebiasis is most difficult to assess inasmuch as a person may have the infection and still not exhibit any symptoms whatsoever, or a patient may have highly suggestive symptoms and the laboratory demonstration of amoebic cysts in the stools may not be forthcoming

Routine examination of the stools for cysts has been carried out in different parts of the world with very variable results While in certain parts of the U S A it has been assessed that as many as 5 to 20% of apparently healthy persons harbour cysts similar studies in Lucknow and in Madras have shown a lesser incidence of cyst passers This is only one example of the vagaries of examinations for amoebic cysts I am not aware of any serological or tissue sensitivity test which may perhaps find more universal application than the demonstration of the amoeba or the cyst in the stool The complement fixation test has not been accepted as reliable

With the limitations in laboratory diagnosis therefore it is not surprising that the clinical diagnosis of amoebiasis is apt to be exaggerated or under estimated according to the fancies of a particular physician

Clinical Types

Acute amoebic dysentery

No detailed description of this common clinical condition is necessary Although the differential diagnosis between severe bacillary dysentery and mild amoebic dysentery can be fairly easily made on clinical data alone such as the number of stools the naked eye character of the stools and the constitutional reactions the distinction is more difficult in the average cases The busy practitioner prescribes phthalyl sulphathiazole on hearing the patient s account of the dysentery and concludes that the case must have been one of bacillary dysentery because symptomatic improvement has been obtained with the sulphonamide It is not sufficiently realised that the activity of the amoeba is intimately related to the bacterial content of the bowels which indeed determines the occurrence of overt symptoms and also makes any definite statement about the incubation period impossible An antibacterial drug like sulphaguanidine or phthalyl sulphathiazole is therefore quite capable of producing a temporary improvement in the clinical picture even in amoebic dysentery But for a permanent cure, entirely different drugs must be used

It is therefore extremely important that in every first attack of dysentery, the

[illegible] well known procedures it is unfortunately almost impossible to obtain data regarding the prevalence of amoebiasis in the community But there is no doubt that as a cause of chronic invalidism leading

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to frequent absenteeism and therefore to curtailment of the man hours put into the national productivity plans amoebiasis is as important as the other diseases mentioned

The frequent neglect of the first attack leads to the patient becoming a

be extremely difficult

(b) In children between the ages of 3 and 10 pronounced anaemia may be caused by chronic amoebic dysentery The number of motions per day is not

When

eveals a

to think

(c) The patient complains of flatulent dyspepsia with perhaps attacks of palpitation but hardly of any other symptoms suggestive of a chronic intestinal pathology The presence of hepatic involvement undoubtedly adds to the frequency and severity of these symptoms

(d) Retention of urine has been the leading complaint in a few cases with lesions in the sigmoid and rectum This symptom is possibly due to a reflex mechanism and among its numerous causes amoebiasis should be remembered

Complications in the liver

(a) *Hepatitis* —*Amoebiasis of the liver is to my mind the commonest cause* of an enlarged liver in South India The picture of amoebic hepatitis is so

cause of a pyrexia of undetermined origin The temperature is usually noticed in the evenings may be preceded by a chill seldom rises above 101 °F and is often not diagnosed properly

The relationship of hepatic amoebiasis to other hepatic diseases

Although the vegetative forms are permitted by lack of proper and timely treatment to reach the liver in most cases of amoebic dysentery the actual occurrence of hepatitis has been estimated to take place only in less than 10% of the subjects Possibly the nutritional status of the liver, which suffers early in malnutrition is an important factor determining the incidence of amoebic hepatitis

rupture into the lung and produce an inflammation which may even go on to abscess formation If, at the time of rupture, pleural adhesions are not present, a state of pyo-pneumothorax can result Pleural effusions containing serous fluid or the typical chocolate coloured pus of amoebic infection, have been noted in certain cases

Apart from such direct extension by actual rupture, a lymphatic extension seems also possible

All these cases where the involvement is usually in the right base and is the result of rupture or lymphatic permeation from the liver have been designated as "secondary pulmonary amoebiasis"

inasmuch as the intestinal infection occurs first in every case

Several types of lung involvement are possible, namely—

(a) Amoebic pulmonary abscess without liver involvement

(b) Pulmonary abscess separated from the hepatic process by intervening normal lung

(c) Pulmonary abscess extending from liver abscess, which is the most frequent type

(d) Broncho hepatic fistula with little pulmonary involvement. Here, communication between the hepatic abscess and bronchus is more direct and large permitting the evacuation of the liver abscess contents with little involvement of the pulmonary parenchyma

(e) Perforation of hepatic abscess into the pleural cavity producing an empyema or a pyo-pneumothorax

I have seen many cases of pulmonary amoebiasis diagnosed on adequate clinical grounds The confirmation of the diagnosis in every case was obtained by the therapeutic response of complete clearing up of all the evidences of the disease, clinical and radiological

The various types of involvement of the lung and pleura have been referred

was rendered difficult by the fact that the sputum examination and the tomograms did not help In view of the past history, a tuberculoma was thought of and in view of his age a carcinoma was also considered A thoracotomy was done The examination of the contents of the segment resected revealed amoeb1

Studies conducted in Madras

15

amoebic origin (Stanley Hospital, 1957 and 1958)

3 Reddı and Thangavelu (1) have analysed 1,011 cases occurring during the 5-year period 1941-46 Of these, 8% were in the age group 0-10 years while the greatest incidence was between 20 and 40 Males formed 82 7% There was a higher incidence in the monsoon months Of the 44 cases which came to autopsy, the majority showed the primary lesion starting in the caecal area The authors had previously reported 6 cases of amoeboma (amoebic granuloma of the large intestine) During the same period 30% of all types of hepatitis were diagnosed to be of amoebic origin

They have also reviewed a series of cases of amoebic liver abscess, 84 from the General Hospital, Madras and 40 from the King George Hospital, Vizagapatam The possible aetiological relationship of alcoholism is discussed They

autopsied at Mysore and cases of cutaneous amoebiasis seen in Madras

4 Tetracyclin (250 mg in capsules) was given 4th hourly for 10 days, along with 1 tablet of vitamin-B complex, in 6 cases of acute intestinal amoebiasis In all of them the vegetative forms disappeared There was clinical improvement in the dysentery in all cases

Chlor tetracyclin, erythromycin and fumagillin are the three antibiotics which are claimed to have a direct action on the amoeba The action of the other broad-spectrum antibiotics in controlling amoebic dysentery seems to be by the indirect method of inhibiting the activity of the bacteria in the intestinal lumen Apparently, *Entamoeba histolytica* cannot assume pathogenecity until the bacterial flora of the intestinal tract reach a certain concentration

5 Entamide (dichloracet-4-hydroxy-N-methylanilide) was tried in 30 subjects passing amoebic cysts and in 33 cases with acute amoebic dysentery

the drug (2)

6 Krishnaswami and Vaidyanathan (3) have reported on a series of 20 patients treated with camoform 1 to 2 tablets thrice a day with meals for 5 days Fifteen of them showed marked improvement *E histolytica* disappeared between

of trophozoites lying free in the lumen of the intestine the amoebae in the wall of the large bowel and what have travelled to the liver and other remote areas, and lastly the cysts in the lumen of the intestine

The other usual considerations in the choice of any drug such as ease of administration freedom from toxicity and acceptability by the patient must also be taken into account

In spite of the continuous search that has been going on there is no single drug which is effective both against the trophozoites in the various sites and the cysts It is, therefore, essential to employ a combination of amoebicides acting in different ways

The schedule of treatment that was considered to be the best until 1948, largely as the result of the experience gathered during the last war was—

Days 1- 6 Emetine injections gr ½ to 1
7 9 Carbarsone gr 4 b d after food
10 15 Emetine injections gr ½ to 1
16 22 Carbarsone gr 4 b d after food
16 25 Chiniofon retention enemata starting with 8 oz of 0 5% increasing by 0 5% each day up to 2 5% repeating 2 5%

It is imperative that every patient should be given the entire course of the treatment if there is to be any reasonable prospect of a permanent cure

avoided in the management of amoebiasis In fact for over three years now, I have not used emetine at all in my practice

The schedule of treatment for the first attack of amoebic dysentery that I now employ is as follows

Days 1 7 Tetracyclin (250 mg) or erythromycin (100 mg) 4 times a day orally
1 14 Chloroquine diphosphate orally 300 mg of the base b d after food for the first 2 days and then 150 mg b d
15 28 Diadoquine entamide or equivalent 600 mg t d s after food

Cases of amoebic hepatitis and pulmonary amoebiasis have shown uniformly satisfactory response to chloroquine In these cases of definitely established

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c

patients have the entire course going about their work normally whereas others complain of all the symptoms enumerated above even after a day s treatment

retinopathy following chloroquine therapy refers only to cases treated with the drug for about three years continuously

The schedule of treatment indicated above is effective in acute amoebic dysentery particularly the first attack Every now and then, however, one meets with a neglected case of chronic amoebiasis involving both the bowels and

the liver and of a fairly long duration which is extremely difficult to manage One such patient with multiple foci of infection including skin had to be given chloroquine I M for six weeks The patient could not tolerate the drug orally but with this long course of chloroquine, he showed remarkable improvement It should have been obviously impossible to keep on emetine for such a long period In some of the cases the chloroquine course had to be repeated three times with intervals of a few weeks in between before a permanent cure could be established The exact duration of treatment therefore has to be individualised

Prevention —The difficult problem of effectively controlling a bowel infection in a country with a poor level of general sanitation cannot be discussed here at any length In the field of tuberculosis I feel that as medical men we cannot sit idle with folded hands waiting for the distant day when the general living [illegible]

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DISCUSSIONS

DR P K GHOSH Calcutta

Q The treatment advocated by you is expensive for an average Indian patient and does not take into account the secondary infection in amoebiasis stressed yesterday

Ans Prolonged treatment is essential to get a permanent cure and to prevent a relapse Less expensive treatment may be less effective too

DR SUBHIE Lucknow

Q What is the youngest age incidence in the series ?

Ans 2 years

DR R T KAKADE Hyderabad (Dn)

Q What was the radiological findings in secondary pulmonary amoebiasis ?

Ans Like lung abscess

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CLINICAL MANIFESTATIONS OF AMOEBIASIS

R M KASLIWAL AND M S GAMBHIR

From the S M S Medical College & Hospital, Jaipur

Amoebiasis is a disease which can have very varied manifestations Boyers (1) reported 1961 symptoms in his 700 patients of amoebiasis Amoebic dysentery is only one of these but to many clinicians this has erroneously become just a synonym of amoebiasis or amoebic infection Craig (2) remarked 'The vast majority of such infections are not accompanied by dysenteric symptoms but much

or physiologically subnormal But as Peterson (8) has remarked, "*E histolytica* lives at the expense of the host and therefore some degree of ulceration of the intestinal mucous membrane is always present"

simulating cholecystitis and another 11 cases had burning pain in epigastrium simulating peptic ulcer In 21 cases (1 1%) there were symptoms of subacute obstruction simulating abnormal tuberculosis and in 5 cases a palpable mass was felt in the regional descending colon simulating a malignant growth These *masses disappeared after successful antiamoebic treatment*

Hepatic involvement —Liver involvement is the most common and the most important metastatic complication of intestinal amoebiasis In many of these cases there may be no clinical manifestation of intestinal phase and hepatic involvement may be the first clinical manifestation Enlarged liver was felt in slightly more than 50% of cases and about 16% cases presented as hepatitis Liver abscess was seen in 2 2% (43 cases)

Small amount of right sided pleural effusion (which could be explained by

developed a bronchopleural fistula and the patient was bringing out anchovy sauce type of material in sputum

These findings confirm the observations of Loeber and D Antoni (9), Sodeman (10) and Kasliwal (6) that liver involvement is a very frequent manifestation in amoebiasis

Anaemia —Slight degree of anaemia was very common but marked degree of anaemia was seen only in very few cases (less than 1%) This confirms the

pyelitis malaria etc

sigmoid colon which is also slightly tender on deep pressure This combination has been found to be useful index for labelling cases of clinical amoebiasis

Summary and Conclusions

Clinical records of 1934 cases of amoebiasis admitted during last 10 years to the S M S Hospital Jaipur, were studied It was seen that most of the cases present bizzare symptoms of vague pain in abdomen and irregularity of bowel habit Important physical findings were a slightly enlarged (tender or non tender) liver and palpable and tender descending colon in large majority of cases

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Discussions

DR P K GHOSH Calcutta

Q What is the pathological basis of the symptoms of cholecystic and headache syndrome in amoebiasis

Ans Headache is possibly due to dilatation of meningeal vessels by toxins (toxaemia) Diagnosis of cholecystitis was made by rapid improvement with antiamoebic drugs About exact pathology I am not sure

Q Therapeutic test is not diagnostic of amoebiasis, for 80% of usual cases are cured by any medicine

Ans No Mere psychotherapy cannot cure or improve definite cases of amoebiasis

DR V S MANGALIK, Lucknow

Q Why is there strikingly low incidence of functional bowel disorders such as diarrhoea, dysentery and constipation etc The total incidence being as low as 23%

Ans Chronic cases may not have any intestinal symptoms

Q Why strikingly low incidence of anaemia in these cases ?

Ans In recording anaemia I have avoided cases with mild anaemia hence the chart shows a low percentage of anaemia

DR J B SRIVASTAV, Kasauli

Q Polymorphs in pleural cavity are not diagnostic of amoebic involvement, because the reaction is mononuclear in amoebic infection and the liver abscess is sterile

Ans *Although theoretically polymorphs should not be in the pleural fluid,*

... you

Ans There was no eosinophilia in my cases

Q Did you come across any asthmatics of amoebic origin?

Ans No

Q Did you ever take the blood pressure ? What are the effects on blood pressure ?

Ans I do not have any definite data about blood pressure But usually in chronic amoebiasis it is slightly lowered

DR SUBHIE, Lucknow

Q What was the youngest age of incidence in the series ?

Ans 9 months

DR S C MISRA, Lucknow

Q Your case records show 80% of patients had thickened sigmoid Have you had your observation confirmed by laparotomy ?

Ans I was quite sure that there was no reflex spasm hence my clinical findings were absolutely correct Moreover, most of the cases were confirmed by sigmoidoscopy

DR K S SANJIVI, Madras

Q Will you kindly tell me the percentage of cases having positive trophozoites and positive cysts in stool?

Ans Cysts 100% trophozoites—great majority

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HEPATIC INVOLVEMENT IN AMOEBIASIS

R M KASLIWAL AND M S GAMBHIR

From the S M S Medical College, Jaipur

Clinically, hepatic amoebiasis exists in two forms (i) amoebic liver abscess and (ii) amoebic hepatitis Amoebic liver abscess is well recognised as a definite pathological entity The occurrence of liver abscess has been known since the time of Hippocrates who is supposed to have advocated a surgical treatment for this condition

Councilman and Lafleur (1) probably gave the first account of pathology and *pathogenesis of amoebic abscess of the liver and in a later work* of Rogers (2) the aetiologic and pathologic basis of this condition was established beyond doubt

In amoebic hepatitis the picture is confusing and it has not yet received the

of *E histolytica* to those who completely deny the existence of any diffuse lesions in the liver in amoebiasis

(3) coined the term 'presuppurative stage' of hepatic amoebiasis for the condition the clinical picture of which he had described in his earlier work (Rogers, 2)

Since this description of Rogers, there has been a lot of controversy with regard to the existence of such a stage as a pathological entity Two schools of thought have however emerged during this period in this field—one which

The evidence in favour of or against the existence of amoebic hepatitis has mainly come from following sources

(i) Results of autopsy studies in cases dying of amoebiasis with or without a clinical evidence of amoebic hepatitis

(ii) Liver biopsy studies in cases of amoebic hepatitis

(iii) Study of liver function tests as an evidence of liver cell necrosis and diffuse lesion

(iv)

Palmer (8) reported autopsy findings in 19 cases whose bowels showed active amoebic lesion Microscopically there was mainly an increase in the portal connective tissue In 9 of these cases there was a definite periportal fibrosis In some of these patchy areas of fibrosis were also seen

Craig (9) also found similar areas of fibrosis in the liver tissue of cases dying of amoebic dysentery Chatgidakis (10) studied liver tissue in 157 cases dying of amoebiasis—out of a total autopsy record of 9 500 deaths In this series, 87 had amoebic liver abscesses and one had acute amoebic hepatitis The findings in this case were very interesting Small areas of necrosis were seen and these areas contained remains of necrosed liver cells and a number of lymphocytes

On the other hand, DaSilva (11) and Donoso *et al* (12) in a review of a large number of autopsies (5 000 by DaSilva and 10,910 by Donoso *et al*) which included a number of cases of amoebiasis did not find any evidence of diffuse hepatic lesion due to *E histolytica* in any case Kean (13) who is a keen supporter of the school which does not believe in the existence of amoebic hepatitis reviewed the records of 4,478 autopsies at the Board of Health Laboratories, Gorgas Hospital, Ancon Thirteen of these cases died of amoebiasis To these he added his own series of 148 cases dying of amoebiasis with clinical evidence of liver involvement He did not find even one case which could meet the criteria for diagnosis of diffuse amoebic hepatitis (He has, however not given the details of what he considers as the criteria for diagnosing this condition)

Liver biopsy studies According to some, autopsy studies may not be considered as true representatives of the tissue changes as seen in living condition and thus, recently, some liver biopsy studies have been made

Bonnin and Moretti (14) gave the first report of liver changes in hepatic amoebiasis as seen by liver biopsy In 4 liver biopsies they found periportal fibrosis and some areas of local inflammatory changes

Heller *et al* (15) performed liver biopsies in two cases of amoebic hepatitis These cases were very interesting in the respect that they were presented as cases of pyrexia of unknown aetiology Routine investigations were negative and they made the clinical diagnosis of amoebic hepatitis Liver biopsies showed areas of focal necrosis Both the cases responded favourably to antiamoebic treatment

any case In a repeat biopsy after the treatment they found that there was a

cell pattern

Chaudhuri and Saha (17) performed liver biopsies in 15 cases of active amoebic dysentery and found some non specific inflammatory changes in some of these cases There was round cell infiltration of the portal tracts and an overactivity of Kupffer cells No amoebae were found in the lesion

Similar liver biopsy studies by workers belonging to the other school of

(13) reported biopsy studies in
ically) He did not find evidence
non specific) in a single case In
41 cases, he labelled the tissue as normal and in 6 found evidence of other associated pathology Three remaining cases had liver abscess

DaSilva and Torres (18) also in a study of 40 cases of chronic amoebiasis, 7 of which were in acute phase of the disease, did not find any specific change in the liver tissues, which they could correlate with amoebic infection

amoebic dysentery In 3 of these cases the functional impairment was considerable Heilig and Visveswar (21) carried out a study of liver function by Quick's hippuric acid test in 15 cases of amoebic dysentery and 11 cases of acute amoebic hepatitis They found that prior to therapy the liver functions were much impaired (average value 55 4% of normal) There was marked improvement *with emetine therapy After 6 injections the average value increased to* 72 6 of normal and after a full course of 12 injections to 80 2% of normal

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have been reported by Cagil and Marinoni (25), Misra and Bajpai (26) and Kasliwal and Sogani (24) in cases of amoebic hepatitis Powel *et al* (27) have also found similar changes in electrophoretic analysis of serum proteins in amoebic hepatitis though they do not attach much importance to these changes

The only report which is contrary to these observations is that of Powell *et al* (27) who performed a number of tests of liver function in 49 cases of amoebic dysentery and 31 cases of liver abscess He found that in all the cases of dysentery where there was no liver abscess, the liver functions were within normal limits and only in the presence of liver abscess, functions showed some derangement

Experimental studies Carrera (28) conducted some experiments in kitten He inoculated 183 kitten per rectum with trophozoites of *E histolytica* Two of

same lines did not get the same type of results They injected trophozoites
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mesenteric and portal veins They found amoebae in such lesion (They have not given the details of histological findings of such lesions) Another important observation in this study, which they noted, was that liver abscess could be produced by injection of *E histolytica* in the portal vein only when there were amoebic lesions in the intestines In the absence of such intestinal lesions no [illegible]

specific liver changes ?

Councilman and Lafleur (1) commenting upon the histiogenesis of the diffuse hepatic changes observed by them in an autopsy in a case of amoebiasis suggested that these changes are due to the absorption of chemical products of amoebae from the intestinal canal

Rogers (3) expressed the view that amoebae reach in the liver become entangled in blood clots in the intralobular veins producing congestion of the liver The vast majority of amoebae then undergo degeneration but produce changes in the liver which he termed pre suppurative stage of amoebic hepatitis

Palmer (8) remarked that in the development of hepatic amoebiasis if an abscess does not form and the process is progressive this terminates in an irregularly scarred liver

Craig (9) stated that the diffuse hepatic lesions represent the foci of infec [illegible]

lytica lying in a zone of completely cytolysed material This may progress either to true abscess formation or healing occurs with connective tissue replacement

According to Faust (31) amoebic hepatitis should be considered as due to multiple small colonies of *E histolytica*, although in most cases the amoebae do not colonize the liver parenchyma but die as the result of amoebostatic action of the liver [illegible]

DeBakey and Ochsner (32) and Blanc (33, 34) have suggested that in the early phase of amoebic hepatitis there is balance between two processes (i) progression to suppuration and pus formation and (ii) regression towards healing by scar tissue

Kashwal and Bhatia (5) expressed the view that the diffuse hepatic changes are caused by *E histolytica* and not by bacterial association They cited in their support the observations of Wahi and Tandon (35) who found similar liver changes in other protozoal infection like malaria and kala azar

[illegible] hepatic lesions are due to *E histo-* [illegible] that [illegible] these

DaSilva and Torres (18) consider that the mild hepatic lesions which correspond to the designation of amoebic hepatitis are due either to the absorp-

tion of bacterial products through the damaged bowel or to some nutritional deficit co-existent with the parasite infection or caused by it Thus, Dunlap *et al* (36) conducted liver biopsies in 88 patients suffering from surgical disorders of abdomen (other than amoebiasis) and found that the lesions are non-specific and there is not much difference in the histological changes even in such diverse condition as peptic ulcer, pancreatitis, cholecystitis etc The lesions described by them resembled those described by other workers (37) in amoebic hepatitis

We started work on the problem of hepatic amoebiasis in 1954 Part of this work has already been reported (4, 5, 24) and the work is still being continued (38)

(hepatitis in 16% cases, abscess in 2 2% cases)

Seeing such a high incidence of hepatic amoebiasis we started liver biopsy in our patients with intestinal amoebiasis with stool repeatedly positive for *E histolytica* A thorough clinical examination was done to find evidence of any associated infection like malaria etc which might affect the liver Biopsy was performed in 30 cases with amoebic pathology only The following histological

in the same zone of hepatic lobule and pyknosis or balooning of nuclei Some patches of cells were anuclear In addition areas of focal necrosis provided another frequent change being present in 21 (70%) cases This consisted of dissolution of a patch of parenchymal cells which were overrun by a motley of

biopsy, in others, several such wide patches were seen The trophozoites of endamoeba described by some workers in experimental amoebiasis were looked for and could not be identified with certainty

Reticuloendotheliosis was present in 28 (93 33%) cases This also varied

contain a light brown pigment

Portal infiltration was seen in 12 (40%) cases The infiltration cells were chiefly mononuclear, although a few polymorphs were also included This change, however, was never severe

Fibrosis portal or intralobular was conspicuous by its absence in all cases but one (3 33%) In this case, the fibrosis was limited to the portal area which was considerably enlarged and included 2-3 proliferating biliary ductules The fibrosis did not extend into the lobule and the normal lobular relationships were still maintained

consisted of cases with no hepatomegaly or slight nontender hepatomegaly Group II (16 cases) consisted of cases of frank amoebic abscess The idea of this classification was to divide cases of intestinal amoebiasis with no clear cut (clinically) involvement of the liver, the second consisting of cases of intestinal amoebiasis with clinical evidence of liver involvement but no demonstrable abscess

cholinesterase activity alkaline phosphatases and serum protein fractionation by electrophoresis were also done

A fall in serum cholinesterase levels was seen in cases of amoebiasis The fall in cholinesterase activity was consistent with the severity of liver damage The decrease was only in 27 2% cases of Group I, 56 2% cases of Group II and in 100% cases of Group III

Alkaline phosphatase levels were normal in all the cases of Group I and Group II but high values were seen in 66 6% cases of Group III

(a) Liver enlarged slightly below the costal margin and tender on pressure
(b) Radiological evidence of hepatomegaly and impairment of movement of right dome of diaphragm
(c) *E histolytica* cyst demonstrated in stool
(d) Response to antiamoebic treatment in the form of disappearance of liver and in general condition

Liver biopsy in these cases showed changes ranging from congestion of portal

infiltration

Liver abscess (4 *cases*) Liver was enlarged and very tender Anchovy sauce pus aspirated out and sterile on culture except, in one case where the initial aspiration contained frank yellow thick pus (about 10 cc and streptococci were cultured from that specimen) followed by anchovy sauce pus sterile on culture *E histolytica* could not be demonstrated in 2 cases even on repeated examinations and by concentration method

[1] Serum glutamic oxalacetic transaminase [2] Serum glutamic pyruvic transaminase

Liver biopsy Taken from an area proximal to the abscess cavity showed all the previous changes and, in addition, showed periportal fibrosis with liver cell pattern disorganisation suggesting chronicity

Transaminase level Normal values established in 40 average Indians out of which 20 were medical students and doctors, 15 were blood bank donors and 5 healthy males with no clinical evidence of any abnormality These values are

(*a*) *Normal values*

S G O T = 4 48 mean 25 units
S G P T 1 35 mean 9 units

(*b*) *Ten cases of amoebic hepatitis*

S G O T = 6 52 mean 21 8 units
S G P T = 1-24 mean 7 7 ,,

(*c*) *Four cases of liver abscess*

S G O T = 20 216 mean 89 3 units
S G P T = 1-145 mean 50 6 ,,

More cases are being studied and the work is in progress

Comments

The different shades of opinions regarding the existence or non existence of diffuse lesions in the liver in amoebiasis may be grouped as follows

(i) There are no diffuse hepatic lesions in the liver (11-13)

(ii) [illegible]

ency anaemia etc [illegible]

(iii) The diffuse lesions in the liver in amoebiasis are due to the chemical products of the amoebae absorbed from the intestinal canal (1)

(iv) The diffuse lesions in the liver are due to large number of amoebae which reach the liver and cause thrombosis of many sinuses and thus necrosis of liver cells The vast majority of amoebae then undergo degeneration (3)

(v) The lesions are due to specific activity of the amoebae and a number of these are found in the liver tissue (9,28,31—34)

Thus, on the one extreme, some workers completely deny the occurence of any diffuse hepatic lesion (other than focal lesions of amoebic abscess) in amoebiasis and think that there is no pathological entity as amoebic hepatitis

On the other extreme, some believe that there are diffuse changes in the liver in amoebiasis and these are due to the specific activity of *E histolytica* in the liver tissue [illegible]

histological changes the occurrence of diffuse reticuloendotheliosis in almost [illegible]

The changes in [illegible]

protein fractionation did show some changes (24) Thus, the lowering of cholinesterase activity in 56 2% of cases of group II (who had no clinically demonstrable liver abscess) indicates that there is some damage of liver cells even in these cases and this may be due to amoebic hepatitis The findings of Heilig and Visveswar (21) also are in the same line and they have shown that the functional impairment shows improvement after successful antiamoebic treatment

fibroblasts The liver cell pattern remains normal except for occasional foci of liver cell necrosis and cellular disorganisation These changes confirm our previous observations (5) In liver abscess cases in biopsy taken from near the

by scar tissue

With regard to changes in transaminase activity it was seen that in amoebic hepatitis the levels ranged from 6 to 52 units of S G O T (mean value 21 8) and 1-24 units of S G P T (mean value 7 7) In 4 cases of amoebic liver abscess the values of transaminase activities ranged from 20-216 units of S G O T (mean value 89 3) and 1-145 units of S G P T (mean value 50 6)

the other findings of amoebic hepatitis stage, there is also a pre-hepatitis stage or

an early hepatitis where the involvement is normal This is probably the same stage which Klatskin (6) has termed as subacute and chronic forms of amoebic hepatitis

From these observations our conclusions are

(a) That there is some degree of hepatic involvement in a very large majority of cases of amoebiasis and Radke (41) has correctly remarked that there is hepatic involvement in almost every case of amoebiasis

(b) That there are three stages of liver involvement in this disease

(i) early hepatitis or prehepatitis stage which is manifested by slight enlargement of liver and minimal or no constitutional disturbances as fever, leucocytosis etc and this stage is the most common of all forms of hepatic amoebiasis and occurs in more than 50% of cases of intestinal amoebiasis

(ii) A definite amoebic hepatitis in which there is hepatomegaly and also constitutional disturbances (it was seen in 16% of cases in our study)

(iii) Amoebic liver abscess occurs only in small number of total cases of amoebiasis (2 2% in our study)

This stage of early hepatitis or prehepatitis can be easily recognised if looked for and will very well respond to antiamoebic treatment Sodeman (43) [illegible] that "[illegible] before the stage of ac[illegible]te hepat[illegible]

another clinical picture of amoebiasis with hepatic change

SUMMARY

A clinical study of the incidence of hepatitis in a large number of cases of [illegible]

[illegible] but bacterial associates alone cannot account for such changes specially reticuloendothelial hyperplasia

[illegible] ded that there is a definite stage of amoebic hepatitis which [illegible] a pre-hepatitis

ACKNOWLEDGMENT

We are thankful to Dr J C Sarin, Reader in Pathology, for helping us in the histopathological study of liver biopsies and Shri I C Sogani of the Department of Medicine, for his help in carrying out estimation of transaminase activities

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(b ... *Med Hr* ,
53 : 190
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36 ... *Obstet* 90 : 220
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43

DISCUSSIONS

Dr J B Shrivastav Kasauli

Q As cellular reaction will be better when the defence reaction is good and not otherwise I think cellular infiltration in your slides is due to resistance

Ans I do not agree with you In my opinion cellular reaction is entirely due to amoebic infection

Dr S H Zaidi Lucknow

Q I think there is no marked cellular infiltration in your slide

Ans No Cellular reaction is of considerable degree in my slides
Dr V S Mangalik, Lucknow

Q Various workers have reported different kinds of reaction in hepatic amoebiasis like monocytic lymphocytic, reticuloendothelial, fibrocytic reaction It may appear these are not specific to amoebic infection but may be non specific depending on other coincidental toxins, etc

Ans Cellular infiltration can partly be due to malnutrition but malnutrition cannot be the sole factor

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PLEURO PULMONARY AMOEBIASIS

R N TANDON AND B K KHANNA

From the Kasturba T B Hospital Medical College, Lucknow

below in detail

CASE REPORTS

I R S aged 22 years, clay-pot maker by occupation was admitted to our hospital on 24 3 58 with complaints of low grade fever, cough, moderate hemoptysis (four times) and pain in right side of chest for last one month The

negative X-ray chest revealed a shadow at the right base suggestive of pneu-

Tomography of the right base, failed to demonstrate the ring shadow Liver receded back

II N, aged 40 years, was admitted to our hospital on 18 12 58 with complaints of cough, low-grade fever, expectoration and hemoptysis at home for last 15 days The patient had developed 15 days back high fever with rigor which came down after a day by itself Thereafter the patient started having excessive dry cough
about 2 oz in amoun
blackish red It was
sputum persisted On examination, right infrascapular area was found to be dull with diminished air entry The liver was not palpable X-ray chest revealed raised diaphragm on the right side with irregular contour In its middle, there

was a rounded elevation On screening the diaphragmatic movements appeared to be restricted Increased reticular markings were visible in right lower zone Another X ray following a diagnostic pneumoperitoneum revealed a nodular swelling in the contour of the liver The patient was afebrile and nontoxic The sputum was found repeatedly negative for *M tuberculosis* Tuberculin test with 5 T U P P D was found to be positive The patient was diagnosed to be a case of amoebic liver abscess which had ruptured through the diaphragm and its contents evacuated out through the bronchi To that effect, the sputum was examined for vegetative forms of amoeba, but was found to be negative The patient was given a course of emetine hydrochloride injections as in the previous case After 10 days, the patient felt much better and cough disappeared Radiologically, the reticular markings at the right base diminished and the patient

III

in our

and pa

attack of diarrhoea after which he had high fever with rigor which lasted for 6 days The fever persisted thereafter He reported to a physician who diagnosed the condition as tuberculous pleurisy and put him on streptomycin 1 g I M I O D and I N H 200 mg per day However even after two months of treatment his condition went downhill He then consulted the senior author (R N T) who diagnosed him to be a case of amoebic liver abscess with right sided empyema (amoebic ?) Chloroquine was started at home which brought his temperature down to normal The patient however continued to complain of severe pain in the right chest and hence was hospitalized In his statement he revealed a past history of *diabetis mellitus* which was controlled with 2 tablets of tolebutamids per day The history of amoebic dysentery was also forthcoming Aspiration of the right pleural cavity brought out anchovy sauce pus which contained vegetative amoeba The stools and sputum were found negative for amoeba The patient was put on a course of emetine and chloroquine and repeated aspiration of the chest His condition improved initially But all of a sudden on the 30th October 1959 he develop d acute cor Pulmonale and died within a span of three hours

Discussion

In such a short series, as that of ours although one may not be justified in drawing definite conclusions yet certain important inferences can be drawn from our study

The duration of illness in our series of cases did not extend beyond three months This observation goes to stress the earlier observations of Chakravarti (4) that the history in these cases is usually short Important complaints in our series of cases were cough (5 cases) fever (5 cases) pain in right chest (4 cases)

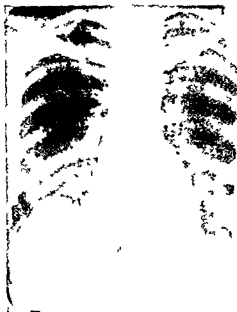
two instances One of these cases was a diabetic

Recent history of dysentery was obtainable in 4 cases In one case inspite of such denial, the incidence of repeated diarrhoea in the past was ascertained Amoebic cysts in the stools could be obtained in only one instance

Tuberculin test with 5 T U was found repeatedly negative in 4 cases This again reaffirms the claim that the presence of negative tuberculin test in hospitalized cases should lead one to suspect non tuberculous conditions



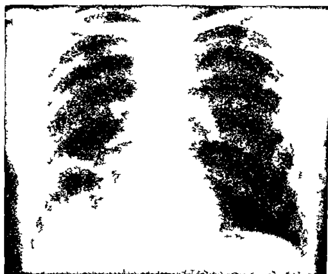
R S
X-ray chest on admission



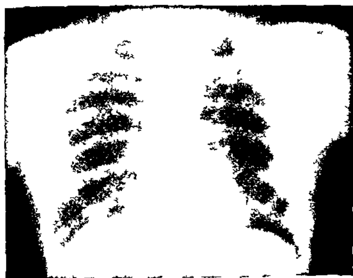
N
X ray chest on admission



R D S
X ray chest on admission



R S
Following therapy



N
X ray chest following therapy

Sputum was found to be negative for vegetative forms of amoeba in our series of cases Chakravarti (4) has also had similar experiences

lowe

cavit

(1 ca

tion

whic

amoebic empyema

The third case (R D S) was a diabetic Not only his condition on admission was moribund he was fairly dyspnoeic also because of the pus filling the whole of the right hemithorax After two or three aspirations, it was found impossible

favour

Abdel Hakim and Higazi (6) have mentioned the following modes of infection of the intrathoracic viscera

(i) Primary pulmonary amoebiasis

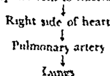
Embolism of amoeba in the bowel reaches inferior vena cava through middle and inferior hemorrhoidal veins This picture appears independently of hepatic involvement and may simulate broncho-pneumonia or miliary tuberculosis

(ii) Secondary pulmonary amoebiasis

The pulmonary involvement in these cases is secondary to hepatic pathology The routes of infection are

(a) direct extension through the diaphragm

(b) embolization from thrombosed hepatic vein to inferior vena cava



In our series of cases, the clinical history and the radiological picture point in favour of secondary involvement of the lungs In 2 cases we have been able to demonstrate clearly the adhesion of liver to the diaphragm In the other cases also this appears to be a potent possibility

Therapy with emetine hydrochloride, 1 gr given by I M I daily for 10 days, has been found to produce a satisfactory response in all but one case who was diabetic and was admitted in a moribund condition

Summary

Five cases of pleuropulmonary amoebiasis admitted to our hospital during the past two years have been recorded. A brief discussion on their clinical feature and response to therapy has been presented

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DISCUSSIONS

DR P K CHATTERJEE, Calcutta

Q Is it necessary to do a diagnostic pneumo-peritoneum in a case of suspected liver abscess bursting into the lungs ? I think there is definite risk of the abscess bursting into peritoneum

Ans I agree There is a possibility of abscess bursting into peritoneum Yet, we did it, to elucidate the fibrous adhesion

DR P K GHOSH, Calcutta

I concur with Dr Tandon that pulmonary amoebiasis is more commonly an extension from liver I had 3 cases under me which all were at right base and above liver But in one of my cases he was treated for 6 months for pulmonary tuberculosis with S M and I N H Finding no relief, he was admitted under me in hospital for treatment He coughed up anchovy sauce fluid in sputum which under microscope showed liver cells It completely cleared up with anti-amoebic treatment He is well till today—8 years after treatment

I do not agree
the case presented w
the lungs up, especi
and (b) the upper l
cavity

Ans I do not think that amoebic liver abscess can push lungs up to such a great extent as shown in the X-ray In the X-ray plate the lung tissue is seen only in the upper zone

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SURGICAL AMOEBIASIS

S G MISRA

From the K G Medical College, Lucknow

The incidence of surgical manifestations of amoebiasis has decreased considerably in recent years It is surprising to note that in a big general hospital like the King George's Hospital, Lucknow, which has over 200 surgical beds and to which about 3500 cases are admitted per year, the number of cases with surgical manifestations of amoebiasis have been less than 50 in the last 5 years Of these, the largest number is made up of cases of liver lesions Lesions of the colon, lungs and skin are uncommon

In this context it may be worthwhile to point out that the surgical manifestations of amoebiasis seem to have a regional basis. While skin lesions are unknown in Uttar Pradesh, they are not infrequent in South India Lesion of the caecal area, called ameboma, is quite common in Bihar and parts of Bengal, while it is rare in U P and Punjab I am merely drawing attention to these facts so that investigations on this possible variation may be undertaken by those interested in intensive research on ameobiasis

Of the lesions in the liver, the commonest observed is liver abscess and next in frequency is hepatitis A study of cases here seems to indicate that the abscess has a greater tendency to point in the epigastrium than other parts

Lung lesions are rarely met with, and it is mostly a lower involvement on the right side secondary to pleural involvement as the result of extension from the liver There was only one case where the lesion was on the left side in lung parenchyma unassociated with any liver lesion

Of the colonic lesions it is surprising that whereas the physicians feel that a large number of cases of amoebic colitis exist, surgical experience at operation and as a result of sigmoidoscopic examination is to the contrary The cases reported by physicians with clinically thickened spasmodic descending colon, at operation do not exhibit any abnormality Similarly sigmoidoscopic examination showing evidence of typical ameobic ulceration are few

During the last five years, 3 cases of granuloma were met with in the distal half of colon and these were labelled as amoebic, because of the good response to specific therapy In conclusion, I would like to stress that thanks to the advances in the medical treatment of ameobiasis the surgical lesions of amoebiasis are becoming infrequent

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ANTI-AMOEBIC DRUGS—A REVIEW

JEAN DRUEY

de La Ciba Societe Anonyme, Basle, Switzerland

the bowels, the intestine also becomes sick, rough and ulcerated. It is a long illness, painful and dangerous If treatment is begun while the body still has

akened

ered to

est was devised by K M Lynch (1915) and was more recently (1937) worked out by W R Jones in particular C Dobell was probably the first (1929) to use monkeys systematically, which were then employed extensively by the school of H H Anderson The cultivation of *Entamoeba histolytica, in vitro* presented great difficulties as it flourishes only in the presence of commensals The first usable

Chemistry was well equipped to make available its large arsenal whenever new biological test methods made their appearance This process began in the 1920 s and continues at a lively rate to day

India may be credited with having discovered for the first time active principles in plants at a very early date which are therapeutically useful even measured by the latest research, from the plant called *Kurchi* which contains conessine as its principal alkaloid

The first anti dysenteric drug to enjoy great popularity was *Ipecacuanha* from South America whose root contains emetine as its main alkaloid It was first mentioned in a travel book by Samuel Purchas that appeared in 1625 From it Pelletier isolated emetine in 1817

The various groups of anti amoebics

accessible to a direct comparison although even here striking differences are often manifest depending on method The chief remaining uncertainty in some cases is whether the effect of
amoebicidal action or rather
of culture associates For th
as the strength of the amoeba
is still more difficult in animal experiments since as yet there is no absolutely satisfactory way of infecting mammals experimentally and particularly because the various species do not produce results that can be compared Above all the therapeutic spectrum in the different animal tests is subject to quite considerable fluctuations Another difficulty in comparative evaluation is the fact that *in vitro* and *in vivo* experiments often diverge greatly so that high activity *in vitro* is of small practical significance On the other hand one may take heart from the fact that almost all anti amoebic preparations which have proved of

the same laboratory

This review includes the following groups of drugs

A Natural products
- *Ipecacuanha*
- *Holarrhena*
- *Simaruba glauca*
- *Brucea* and other plant principles
- Antibiotics

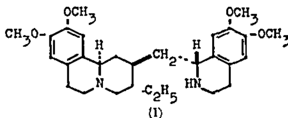
B Synthetic products
- Emetine analogues long chain diamines
- Quinoline and acridine derivatives
- Arsenicals
- Chloracetamides
- Miscellaneous structures
- Phenanthroline derivatives

Special emphasis will be given to the last group of phenanthrolines developed in the last ten years in our laboratories

A Natural products

(a) *Ipecacuanha group*

Emetine (I) is still widely used for the hepatic form of amoebiasis, although Anderson, in a review article in 1950, stated 'that its toxicity precludes its recommendation at present' Its usefulness is seriously limited by its cumulative properties its cardiotoxic effects and its narrow margin of safety Emetine served as a standard for comparison of new drugs, and its chemical structure served as a model for the development of similar compounds



(I)

A new total synthesis of natural emetine in its true stereo chemical configuration was described by O Schneider and Collaborators of Hoffmann La Roche in Basle *There is no doubt that emetine belongs to the true amoebicidal drugs i e* to those which act directly on the amoeba and not by killing the symbionts For this reason it was and still is useful in hepatic abscess, where there are no

Neipp, 1958) was found to be 5 mg /kg during 5 days initiated the rat test, worked with doses therapy In his monkey experiments,

was even more active than emetine *in vitro* and in the monkey

(b) *Holarrhena*

This plant as already mentioned is the oldest of the amoebicidal drugs In Sanskrit it was called Indrayava' or Vatsaka" but it became especially known as Kurchi Conessine is the main alkaloid The biological action of conessine was extensively studied by Chopra in 1927 in a classical paper Favourable clinical results were published by Acton and Chopra in 1933 Later on French scientists became especially interested in this drug It has a lower activity than emetine and is reported to be toxic

(c) *Simaruba glauca*

A comprehensive review on glaucarubin has been given by A C Cuckler in 1958 Chemically this compound was described by F A Ham *et al* * as having

* *J Amer Chem Soc*, 1951, 76 : 6066

the formula $C_{25}H_{36}O_{10}$, containing a lactone grouping, an ester group and 6 hydroxyl groups *In vitro*, it proved to be about 2 to 8 times less active than emetine, using the same testing procedure Its action on amoebae is direct The curative dose in rats according to Cuckler, is 25—50 mg /kg /day Glaucarubin is also active in guineapigs, where it proved to be one of the best amoebicidal drugs For dogs, it is rather toxic but the doses for curative effects in colitis are also much lower The therapeutic dosage for man was found to be 3 5 mg /kg /day for ten days *i e* a total dose of 2 to 3 g

(d) *Brucea and other plant principles*

is claimed to have amoebicidal properties *in vitro* and *in vivo* on the rat (M S Kiang *et al* , see *Chem Abstr* , 53· 11646)

(e) *Antibiotics*

Many antibiotics have been reported as having anti amoebic properties

TABLE I

In vitro *amoebicidal activity of antibiotics**

Group I, amoebicidal	at 5 γ/cc
Actidione	Fumagillin
Anisomycin	Prodigiosin
Group II, amoebicidal	at 63 to 300 γ/cc
Chlortetracycline	Puromycin
Oxytetracycline	
Group III, amoebicidal	at 500 to 1000 γ/cc
Azaserine	Streptothricin
Carbomycin	Tetracycline
Chloramphenicol	
Group IV, amoebicidal	at 1000 to 2000 γ/cc
Bacitracin	Viridogrisein
Neomycin (?)	
Group V, not amoebicidal	at 2000 γ/cc
Streptomycin	Penicillin G
Dihydrostreptomycin	Antibiotic PA 105
Erythromycin	

* According to Thompson *et al* (1956), *Antibiotics & Chemotherapy* 6 : 346

TABLE II

*Comparison of antibiotics on the basis of relative potency in laboratory tests and over-all appraisal as potential drugs for intestinal amoebiasis**

Drug	Relative ratings†			
	Activity *in vitro*	Activity in rats	Activity in dogs	Potential usefulness in human intestinal amoebiasis
Actidione	I	I	I	C
Azaserine	III	V		C
Anisomycin	I	II		A or B
Bacitracin	IV		IV	B
Carbomycin	III	IV	IV	B
Chloramphenicol	III	V	IV	B
Chlortetracycline	II	III	III	A
Dihydrostreptomycin	V	V		B
Erythromycin stearate		III		A or B
Fumagillin	I	I	I	A
Neomycin	IV	IV		B
Oxytetracycline	II	II	III	A
PA 105	V	III	IV	B
Penicillin G	V	IV	IV	B
Prodigiosin	I	II		C
Puromycin	II	II	II	A or B
Streptomycin	V	V		B
Streptothricin	III	II	II	C
Tetracycline	III	IV	IV	B
Viridogrisein	IV	III	III	A or B

* According to Thompson *et al* (1956), *Antibiotics & Chemotherapy*, 6: 246

† I through V represents order of decreasing potency A, potentially adequate alone, B, probably useful mainly as adjuncts to more specific antiamoebic drugs, C, essentially ruled out by toxicity

Five groups and four groups, respectively, were also formed according to activity in rats and in dogs An overall appraisal results therefrom, as seen in Table II Interesting compounds like actidione, prodigiosin and streptothricin were ruled out, especially because of their toxicity Others, marked with an "A" in the table, like fumagillin, proved to be clinically very useful, but toxic side reactions have since hindered their general use in humans The same is true for the high-ranging puromycin Terramycin and aureomycin remain for treatment of amoebiasis Experimentally, rather high doses of aureomycin—100 mg /kg /day—are needed to cure rats in our experiments, *i e* higher doses than of simple synthetic drugs like phenanthroline-quinone, to which we shall refer later

Another antibiotic might be added to the table of Thompson, namely spiramycin, which was discovered independently in our laboratories and by [illegible]

the formula $C_{25}H_{36}O_{10}$ containing a lactone grouping an ester group and 6 hydroxyl groups *In vitro*, it proved to be about 2 to 8 times less active than emetine, using the same testing procedure Its action on amoebae is direct

is 25—50 mg /kg /day Glau

oved to be one of the best amoe

the doses for curative effects in colitis are also much lower The therapeutic dosage for man was found to be 3 5 mg /kg /day for ten days, *i e* a total dose of 2 to 3 g

(d) *Brucea and other plant principles*

Several other plants have been reported as containing anti amoebic principles The *Brucea sumatrana* tree has to be especially mentioned, the fruits of which are used It contains the Chinese anti amoebic Kosam or Ya tan tzu, the active principle seems to be a glycoside called yatanoside A white amorphous compound with glycosidal properties was described by C Y Sung in 1949 ("brucealin") An *in vitro* activity somewhat less than that of emetine is reported Other plants of interest may be the *Stephania rotunda* and *S hernandifolia*, the roots of which are used in India against dysenteric illnesses

In a recent paper by Chinese authors, a root extract of *Anemone chinensis* is claimed to have amoebicidal properties *in vitro* and *in vivo* on the rat (M S Kiang *et al*, see *Chem Abstr*, **53**: 11646)

(e) *Antibiotics*

Many antibiotics have been reported as having anti amoebic properties

ded a large number of antibiotics into several *in vitro* as well as in animal tests (Table I) this last animal for the evaluation of drugs

TABLE I

In vitro *amoebicidal activity of antibiotics**

Group I amoebicidal	at 5 γ/cc
Actidione	Fumagillin
Anisomycin	Prodigiosin
Group II amoebicidal	at 63 to 300 γ/cc
Chlortetracycline	Puromycin
Oxytetracycline	
Group III, amoebicidal	at 500 to 1000 γ/cc
Azaserine	Streptothricin
Carbomycin	Tetracycline
Chloramphenicol	
Group IV, amoebicidal	at 1000 to 2000 γ/cc
Bacitracin	Viridogrisein
Neomycin (?)	
Group V, not amoebicidal	at 2000 γ/cc
Streptomycin	Penicillin G
Dihydrostreptomycin	Antibiotic PA 105
Erythromycin	

* According to Thompson *et al* (1956), *Antibiotics & Chemotherapy*, **6**: 346

TABLE II

*Comparison of antibiotics on the basis of relative potency in laboratory tests and over all appraisal as potential drugs for intestinal amoebiasis**

Drug	Relative ratings†			
	Activity *in vitro*	Activity in rats	Activity in dogs	Potential usefulness in human intestinal amoebiasis
Actidione	I	I	I	C
Azaserine	III	V		C
Anisomycin	I	II		A or B
Bacitracin	IV		IV	B
Carbomycin	III	IV	IV	B
Chloramphenicol	III	V	IV	B
Chlortetracycline	II	III	III	A
Dihydrostreptomycin	V	V		B
Erythromycin stearate		III		A or B
Fumagillin	I	I	I	A
Neomycin	IV	IV		B
Oxytetracycline	II	II	III	A
PA 105	V	III	IV	B
Penicillin G	V	IV	IV	B
Prodigiosin	I	II		C
Puromycin	II	II	II	A or B
Streptomycin	V	V		B
Streptothricin	III	II	II	C
Tetracycline	III	IV	IV	B
Viridogrisein	IV	III	III	A or B

* According to Thompson *et al* (19[illegible]6) *Antibiotics & Chemotherapy*, 6: 346

† I through V represents order of decreasing potency A potentially adequate alone B, probably useful mainly as adjuncts to more specific antiamoebic drugs, C, essentially ruled out by toxicity

Five groups and four groups, respectively, were also formed according to activity in rats and in [illegible]
Table II Interesting [illegible]
were ruled out especi[illegible]
"A" in the table, like [illegible]
side-reactions have since hindered their general use in humans The same is true for the high ranging puromycin Terramycin and aureomycin remain for treatment of amoebiasis Experimentally, rather high doses of aureomycin—100 mg /kg /day—are needed to cure rats in our experiments, *i e* higher doses than of simple synthetic drugs like phenanthroline quinone, to which we shall refer later

Another antibiotic might be added to the table of Thompson, namely spiramycin, which was discovered independently in our laboratories and by Rhone-Poulenc In our hands it proved to be active in rats at a dose as low as 10 mg /kg /day In man, too, it seems to be quite effective

The extremely high potency *in vitro* of compounds like fumagillin and actidione, displaying amoebicidal activity in concentrations of less than 0 1 mg

per liter or approaching a dilution of 1 100,000 000 is worthy of special attention Actidione is the most active compound tried in rats in our laboratory The minimal effective dose was as low as 0 5 mg /kg /day, while for fumagillin this figure was about 4 times higher

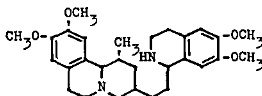
B Synthetic products

(a) *Emetine analogues Various diamine type structures*

Ever since the early days when chemical synthesis was applied to the search for new drugs, compounds closely similar to biologically active natural products as well as compounds resulting at random from the chemist's work have been screened for drug properties

In the search for anti amoebic drugs too we encounter both types of app roach The purely synthetic product not related to any natural product, was developed first, *i e* chiniofon or *yatren* which will shortly be discussed

The first experiments with synthetic emetine relatives go back to the late twenties when a tentative structural formula for emetine became known and when screening possibilities for anti amoebic activity were available Pyman in 1927 attributed the following formula (II) to emetine

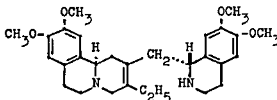


Emetine according to Brindley & Pyman, Soc. 1927, 1069

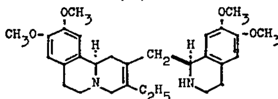
(II)

Pyman prepared *inter alia* a compound composed of two dimethoxytetrahy dro*iso*quinoline groups linked by a 5 carbon chain in 1 1 -position, a logical consequence of his emetine formula It was devoid of anti amoebic activity, as were the analogues with a linking chain of 4 and of 8 carbons According to the correct emetine formula there should have been 3 carbons only Compounds of this type were prepared later, in 1952 by Fulton, but even a product lacking only one carbon atom of emetine was only slightly active (*in vitro*) How specific the structure of emetine is has recently been shown by our colleagues from the Roche laboratories in Basle The racemic 2 dehydro emetine (III) proved ... the stereoche mically ... ivity A ... been synthe- sised and tested

The length of linking carbon chains was varied in Goodson (1948) and in Osbond (1951) Osbond (1959) is a de ethylemetine, it is inactive Some representatives of the type of Goodson (1948) were claimed to be active in rats The diamine type of Goodson 1948 (secondary amines) and Hall 1952 (primary amines) are already far away from emetine the aromatic rings having become simple alkyl chains Already in 1937 Pyman claimed amoebicidal properties for the simple tertiary diamine



rac. 2-dehydro-emetine
highly active
(III)



rac. 2-dehydro-iso-emetine
inactive
(IV)

Dhar and Mahboob prepared aliphatically substituted diamines and these were tested *in vitro* by Kaushiva The most active compounds were found with a central chain of 9 carbon atoms with morpholine or piperidine as a substituent on one side and a hexyl or dodecylamine, the N being secondary, on the other side

Coming back to products having a closer relationship to natural products, I have to refer to the interesting quinine relatives presented here in Lucknow at last year's Symposium on Chemotherapy by Popli, Dhar and Bhandari (Chart 2) It is interesting to note, that desoxyhexahydroquinine, in which the two nitrogen atoms have about the same distance as in emetine is active in intestinal amoebiasis However, the stereochemical isomer of the quinidine series was inactive The second compound has also been described as possessing *in vivo* activity

(b) *Quinoline and acridine derivatives*

To this group bel

of amoebiasis, the

also well-known as chi

properties was quite

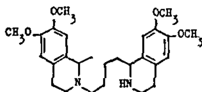
man

knov

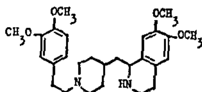
The origin of these iodine-containing compounds lies in the disinfectant virtues of iodine itself, and it was hoped to achieve improved properties by incorporating the iodine in organic molecules It is not surprising, therefore, that the di-iodo-analogue of vioform, diiodoquin, had a halo of efficaciousness when it was recommended for the treatment of amoebiasis in 1936 by A C Tenney

The three drugs are shown in (Chart 3) Clinically they are useful in intestinal amoebiasis, but not in the hepatic form

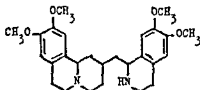
Chart 1



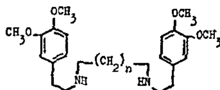
J.M Osbond 1951



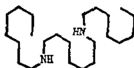
J M. Osbond 1959



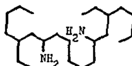
J.M. Osbond 1959



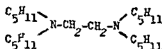
J A. Goodson 1948



J.A Goodson 1948

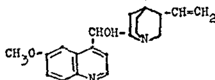


D M Hall 1952

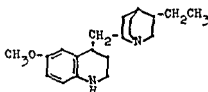


F L Pyman 1937

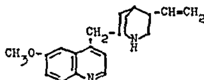
Chart 2



Quinine

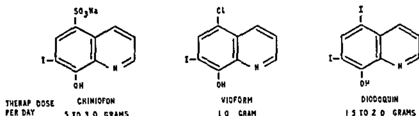


9-Desoxy-hexahydro-quinine
Popli et al. (1958)



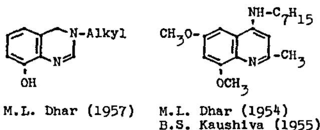
α-[3-Vinyl-piperidyl-(4)]-
γ-[6-Methoxy-quinolyl-(4')]-
propane
Popli et al. (1958)

Chart 3



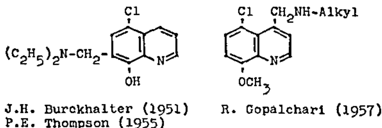
The halogenated hydroxyquinolines being the first synthetic anti amoebic drug, it is somewhat surprising that they were not followed by a large *series of congeners with similar or better therapeutic properties* Several hydroxyquinolines have been tried they were tabulated by Thompson (*Amer J Trop Med Hyg*, 1955 4: 242) Data on analogues of other heterocyclic derivatives are very rare At this Institute this problem with 8 hydroxy quinazoline derivatives was considered (Chart 4) They do not seem to have shown outstanding properties

Chart 4



The 6 8 dimethoxy quinaldine derivatives should be mentioned here too,

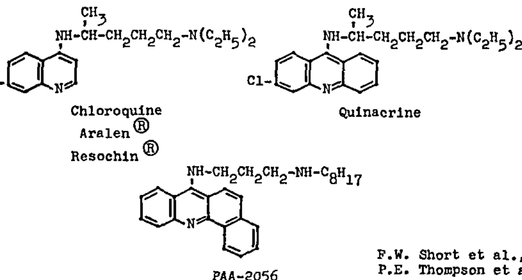
Chart 5



The *in vitro* activity compares favourably with emetine *In vivo*, however, rather high doses are needed to cure intestinal amoebiasis in rats I am not aware of as to whether clinical results have been published Dozens of analogues were prepared and tried in animals In the hamster the compound is probably

too rapidly metabolized to be active systemically In the Chart 5 another

Chart 6



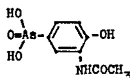
In vitro amoeb cidal conc 2 5-20γ/cc Rats 150—200 mg /kg /day for 90—100 p c cure Dogs 10—20 mg /kg /day for 2 weeks Hamsters 12 5—50 mg /kg /day for 4 days

Quinacrine too belongs to this group From a very large number of similar compounds prepared and tested at the laboratories of Parke Davis & Co in the U S the one called PAA 2056 seemed to be the most interesting *In vitro*, PAA 2056 is almost as potent as emetine (depending on the test conditions) It has a direct effect on the amoebae It is moderately effective against symptomatic intestinal amoebic dysentery in dogs and roughly 8 times as active as chloroquine against hepatic amoebiasis in hamsters It looks promising

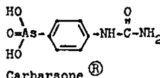
(c) *Arsenicals*

The chloroquine type of compounds brought us back chemically to the diamine type structure They were developed however, by following a completely different way than was taken with the emetine analogues the starting nine Atoxyl was prepared in 1923 The n in Chart 7 ably still the most commc effects By reaction of its trival BAL, balarsen is obtained to compounds goes back to a hypothesis of Voegtlin in 1925 that a senic ... s react with reduced

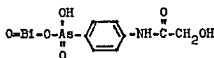
Chart 7



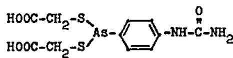
Acetarsone (Stovarsol ®)



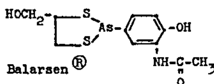
Carbarsone ®



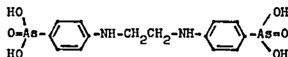
Glycobiarsone (Milibis ®)



Thiocarbarsone ®



Balarsen ®



Diphetarsone

glutathione The first thioarsenites were prepared in the early thirties, but especially H H Anderson followed up this idea further after World War II (1947)

Milibis (R), generic name glycobiarsone, is a mixed arsenical bismuth-organic compound, found in Germany in 1943 (Hauer) It is not resorbed from the intestine and is used mainly in combination with chloroquine

In our hands carbarsone (R) and especially milibis (R) were weak amoebicidal drugs Comparative activity of arsenicals and other amoebicidal drugs is shown in Chart 8

Chart 8

	In rats min eff dose mg /kg /day	in humans g per day
Emetine	5	0 06
Fumagillin	*2*	*0 01 to 0 05*
Aureomycin (R)	100	2 0
Chloroquine	100	0 9 to 0 3
Carbarsone (R)	250	0 75
Milibis (R)	1000	1 5
Entobex (R)	50	0 3
Vioform (R)	100	1 0

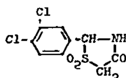
The trivalent arsenic derivatives in this series too were more effective than the pentavalent.

(d) *Haloacetamides*

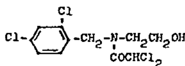
This group (Chart 9) merits being discussed separately as another class of chemicals which went to clinical application from the laboratory desk of a chemist It is a typical example of fortuitous detection of biological activity in a certain compound out of thousands screened every year The starting point of this series of chloroacetamides was the observation that a dichlorophenylthiazolone derivative showed some activity in naturally infected hamsters (*Endamoeba criceti*) In pursuing this observation, many similar compounds were prepared and

related to camoquine, chloroquine and to quinacrine The simple pyridine derivative shown are proved to be the most effective of this series It is also active against amoebic dysentery in dogs but ineffective against amoebic hepatitis in hamsters The Logemann variation with *p* nitrophenoxy is claimed to be active in man

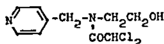
Chart 9



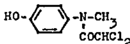
Surrey, 1954



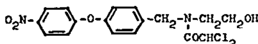
Win 5047 Mantomide ®



Elslager, 1956



Entamide ®

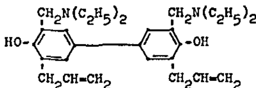


Logemann, 1958

(e) *Miscellaneous products*

This heading does not refer to catch all group It exhibits a drug with

properties were published by Thompson in 1955 Its activity in rats is not outstanding In our hands, too, it was about three times less effective in the rat-test than known drugs such as vioform or aureomycin However, many of the

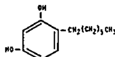


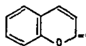
Biallylamicol, PAA 701, SN 6771
Camoform®

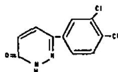
V

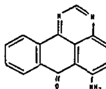
antiamoebic drugs in clinical use have shown that, on a weight basis, from 2 to 70 times as much drug is required for rats as is used in man. The clinical dose of camoform(R) is 1 to 1.5 g. a day for at least five days. On a weight

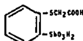
Chart 10

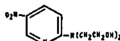


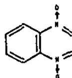


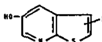


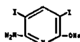














basis it is therefore some ... little ...
line,

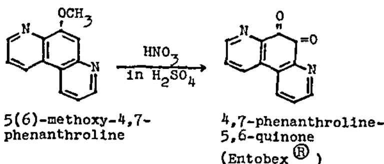
com
publ
have summari compounds tested *in vitro* or in animals for amoebicidal properties In Chart 10 a few structures which have already emerged from the chemical side and claimed to have *in vivo* activity, are given

(f) *Entobex*

A member of this new group, 4 7 phenanthrolinequinone, became known in this country through several clinical publications

Chemically, the phenanthroline derivatives, with which we started with the aim of finding new therapeutic potentialities are based on our experience with the 8 hydroxyquinolines and especially vioform(R) The hydroxy-quinolines are synthesized by the so called Skraup process starting with nitro or aminophenols With diamino as well as with nitro- aminophenols one gets, by a

Chart 11



two fold Skraup reaction the phenanthroline derivatives either directly or one can proceed step by step, obtaining first an amino hydroxy quinoline derivative and by a second Skraup reaction phenanthroline The methoxy 4 7-phe-

anti amoebic drugs in common use has been discussed already Summing up the biological characteristics as they have been determined by Kradolfer and Neipp, the following may be of interest

The ... activity is at least equal or superior to that of emetine

3 In rats, using the Jones procedure, the minimal effective dose (100% cured) was 50 mg /kg The ratio of effective to toxic dose is 1 110, *i e* 30 times greater than with emetine

4 The phenanthroline-quinone is excreted in urine and in the bile in an amoebicidal form Semiquantitative chemical analysis seems to indicate that the

plays a contrary effect, *i e* a bacteriostatic action on colon bacilli

I should like now to give a summary of structure activity relationships in the large number of phenanthroline quinones investigated biologically in our laboratories

In Chart 12, I should like to draw your attention to the three isomeric phenanthrolines, the *o*-,*m* and *p* or 1 10 , 1 7- and 4 7-phenanthrolines

Chart 12

Mono-substituted and unsubstituted phenanthrolines

Compound	in vitro	in vivo	Compound	in vitro	in vivo
N, N	±		N, N	±	-
OH, N, N	++	+	OCH_3, N, N	+	0
OCH_3, N, N	±	-	N, N	±	-
Cl, N, N	+	0	NO_2, N, N	±	-
OCH_3, N, N, CH_3	+				

Without further substituent all the three are almost devoid of antiamoebic properties The only compound showing some activity in the mono substituted group is the hydroxy -4 7-phenanthroline

Among the quinones (Chart 13), the one of the 4 7-series proved to be the most active compound, called

Chart 13

Phenanthroline quinones

Phenanthroline quinones	in vitro	in vivo
	++	++
	+	+(+)
	+	±
	++	++
	++	++

It is an interesting fact that the hydrogenated effectiveness we investigated vn in the litera- advantages over the free quinone

Cyclic derivatives were also broadly investigated I have selected some of them in Chart 15

By cyclization with ethylene diamine, three types of compounds are obtained, the dihydro- or tetrahydro-derivatives or the fully aromatised derivative as a result of some oxido reduction process None of the cyclic derivatives showed outstanding properties so far as the anti-amoebic activity is concerned Some compounds proved to be of interest as anti-tumour agents

Chart 14

Derivatives of 4,7-phenanthroline quinone

Compound	in vitro	in vivo	Compound	in vitro	in vivo
NOH, =O	+	+	$NNHCONH_2$, =O, CH_3	0	±
NOH, =NOH	+	0	NOH, =O	±	
$NNHCONH_2$, =O	++	+(+)	$NNHCONH_2$, =O	+	+

Chart 15

Cyclic derivatives of phenanthroline quinones

Compound	in vitro	in vivo	Compound	in vitro	in vivo
	±	-	H, =O	0	0
HN, NH	+	+	$N(CH_3)_2$	±	0
	±		HN, NH	++	+
SH	0	-			

Annals of Biochemistry
and Experimental Medicine
Vol XX, 1960, Supplement

ENTAMIDE

P T MAIN N W BRISTOW P OXLEY, T I WATKINS G A H WILLIAMS
E C WILMSHURST AND G WOOLFE

From the Med cal and Research Departments Boots Pure Dr g Co Ltd , Nott ngham England

Methods

The methods used in our research laboratories to test new amoebicidal drugs have been described by Woolfe (1956) Large numbers of chemical compounds are screened and, if activity is noted in any compound, then modification of the molecule is undertaken in order to increase activity and decrease toxicity

In vitro *tests*

Drugs which have proved to be active clinically must be active in such a test We use a microtube method in which serial tenfold dilutions of the drug under test are incubated in capillary tubes with strain M of *Entamœba histolytica* in an all-liquid medium (liver extract marmite, and horse serum in phosphate buffer at *p*H 7 2) with rice starch The tubes are completely filled with liquid, plugged with placticine, and incubated in a horizontal position so that multipli

in Table I

TABLE I

Amoebicidal activity—in vitro *microtube method*

Drug		At concentrations of
Penicillin	Inactive	1 in 1,000
Streptomycin	Inactive	1 in 1 000
Chlortetracycline	Active	1 in 10 000
Oxytetracycline	Active	1 in 10 000
Iodo 8 hydroxyquinoline	Active	1 in 10 000
Emetine hydrochloride	Active	1 in 500 000
Entamide	Active	*1 in 10 000 000*
Fumagillin	Active	1 in 100 000 000

It would appear that an antibiotic such as fumagillin has direct effect on the amoebae, whereas other antibiotics have little or no direct amoebicidal action It must be emphasized that this *in vitro* test only indicates which compounds require further study and the ratios of activity for compounds may be quite different in an *in vivo* test

In vivo *tests*

Newly weaned rats are used They are inoculated intracaecally with trophozoites of *E histolytica* dosed daily for five days with the drug under trial and the state of the caecum of each rat assessed on an arbitrary scale The average degree of infection (ADI) for each group can be calculated from the

We

which

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e "just active" dose makes them appear very active They do, however, have a poor dose response curve and high doses are required to completely clear the rats of infection

Results

Using the methods described, a large number of synthetic compounds of various types were screened It was found that a series of substituted acetanilides

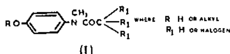
TABLE II

Amoebicidal activity—in vivo (*rat test*)

Drug	Just active dose (mg /kg daily for 5 days)
Carbarsone	250
Iodochlorhydroxyquinoline	150
Entamide	10
Fumagillin	3
Chlortetracycl ne	2 5
Oxytetracycl ne	1 0

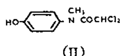
had considerable activity *in vitro* and some were active *in vivo* in the rat The

A preliminary note on entamide was published by Bristow and others (2)



(I)

ENTAMIDE (D OXAN DE)



(II)

Toxicity studies in animals

In view of the high activity of the compound extensive toxicity tests were put in hand by our colleague, Dr M R Gurd The acute LD 50 of entamide orally to mice was about 5 5 g per kg body weight Rats were dosed daily for several weeks with different dosage levels of entamide and apart from some growth reduction and slight liver and kidney enlargement in 1 g /kg daily, there were no toxic effects In cats, apart from occasional vomiting at 1 g /kg daily, the compound appeared to be completely non toxic

Human toxicity studies

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or
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Clinical trials of entamide

Our laboratroy st id es demonst ated that en — d ,
considerable activity
volunteers had reassu
should be submitted to clinical trial

The first pilot trial was undertaken at the Hospital for Tropical Diseases in London by Professor A W Woodruff This unit is well suited for carrying out this type of trial not only because it is particularly interested in amoebiasis but also because the follow up of patients is relatively easy and the problem of reinfection does not occur

Woodruff Bell and Schofield (3) have published the results of this trial They started their trial using a very low dosage of 2 mg per kg body weight daily for 10 days for the r ea l na en and h

treated with 12 mg or more per kg body weight daily—which gives a relapse rate of 11 8% Entamide was found to be most effective in those cases with *E histolytica* (large race) cysts in the stools—only one case relapsed out of 22 treated (4 8% relapse rate)

On the basis of this trial the drug was recommended for further trial as a therapeutic agent for chronic cyst passing patients We have not recommended its use alone in acute dysenteric amoebiasis

A further trial was reported on by Foll (4) in employees of the Burma Oil Company and their dependants Over 30% of all prospective employees were found to have amoebiasis and the vegetative form was the commonest type of *E histolytica* in the stools Foll used a higher dosage of entamide than Woodruff and his colleagues and his dosage ranged from 20 56 mg per kg body weight daily for 10 days Some were treated as in patients and some as out patients Fifty five patients were treated (49 with vegetative forms and 6 with cysts) The relapse and/or reinfection rates of in patients and out patients were as follows

	In patients	Out patients
At 1 month	11 7%	24 3%
At 2 months	23 2%	40 5%
At 3 months	41 2%	64 9%

By comparing a series of patients treated with emetine bismuth iod de in Burma with a similar series treated in London by Woodruff and his colleagues (3) Foll

have reported on their trials of ruff and his colleagues (3) that by about the 5th day of treat They employed three dosage weight daily for 10 days Nine owed up and in only 1 case was there a recurrence, and this was 6 months after treatment and the cysts promptly

disappeared on 40 mg per kg daily Five out of 6 cases on 25 mg per kg daily were followed up, and 1 relapsed at 1 month and was retreated successfully

entamide alone

Apart from the published trials which we have mentioned above, entamide has been the subject of trials in the U S A Kenya, Sudan, the Persian Gulf, South Africa, Malaya, Honduras, and Argentina, and in a few other centres in India As some of the clinicians concerned either have papers in the press or do intend to publish in the future, we will not mention individual trials, but we can say that in 663 well documented cases which have been treated with entamide the percentage cure-rate at the present time in non acute amoebiasis is 72 2 The following tables indicate results in non acute amoebiasis and in acute amoebic dysentery

TABLE III

Entamide trials—non acute patients

Trial	No treated	Cured (%)*	
Woodruff Bell and Schofield (1956)	45	75%	(88% of those given 12 mg /kg / day or more)
Sanjivi and Thiruvengadam (1958)	30	87%	
Toll (1957)	55	42 6%	(54 6% if reinfection rate is 12%)
Toll and Game (1959)	24	43 5%	In patients
	53	34 6%	Out patients
Unpublished trials	436	75 8%	(12 centres)
Total	663	72 2%	

* The % cured is expressed as a % of those followed up and not of those treated

TABLE IV

Entamide trials—acute dysenteric patients

Trial	No treated	Cured (%)*
Sanjivi and Thiruvengadam (1958)	33	51 5%
Other trials	18	66%
Total	51	57%

* The % cured is expressed as a % of those followed up and not of those treated

Absorption and excretion

Wh le these tr al — absorp

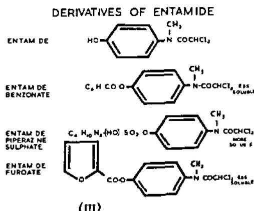
tion und for

estim to use

material tagged with ^{14}C We found that in rats less than 20% of the drug given appeared in the faeces, most was excreted in the urine The drug was in fact absorbed very rapidly from the stomach as well as from the intestine, but there was no accumulation of radioactivity in any organ or tissue Kidney levels were high for a few hours only after dosing, and liver levels were always low

Derivatives of entamide

The rather disappointing clinical effect in acute amoebiasis made us look further at the series We were not sure whether the lack of clinical activity was due to too low concentration of entamide in the lumen of the gut because of absorption higher up in the gastro intestinal tract or whether the blood levels were too low to bring an effective concentration of entamide to the amoebae in the ulcers Accordingly, we prepared derivatives of entamide with both high solubility (and the probability of rapid absorption) and even lower solubility than entamide (to produce a higher concentration in the lower bowel)



(III)

These derivatives were tested in the laboratory and two were chosen at this stage for clinical trial One, entamide piperazine sulphate, was readily soluble in water The other, entamide benzoate, was considerably less soluble than entamide itself Both derivatives were highly active in the laboratory tests The piperazine sulphate was about as active as entamide itself and the benzoate rather more active Both were of low toxicity and therefore suitable for clinical trial We also decided to try entamide in the form of enteric coated tablets

Clinical trials of entamide benzoate and entamide piperazine sulphate

Foll and Game (6) have published some results of trials of these derivatives in Burma, and their paper also reports on an extension of the number of cases treated with entamide itself (Table V)

TABLE V

Drug	In patient or Out patient	No treated	Percentage relapse or reinfection		
			1 month	2 months	3 months
Entamide 20 56 mg /kg /day	IP	24	21 7	47 8	56 5
	OP	51	28 8	48 1	65 4
Entamide benzoate 30 50 mg /kg /day	IP	30	22 2	37 9	50 0
Entamide benzoate 50 mg /kg /day	OP	11	33 3	40 0	70 0
Entamide p perazine sulphate 50 mg /kg /day	IP	30	23 3	36 7	36 7
	OP	5	20 0	40 0	40 0
E B I 3 gr three times daily	IP	12	16 7	33 3	33 3

Foll, and Game, (6)

These results again show a much higher relapse rate for entamide than do those of other workers but it can be seen that the relapse rate with entamide p

th

ar

effective than the parent drug Preliminary results from other workers indicate that enteric coating does not improve the efficiency of entamide

Entamide furoate

While these trials were in progress experimental work continued with the main emphasis on esters of entamide From the many esters made, the furoate was outstanding in its activity, being several times more active than entamide in rats Toxicity studies showed that it was even less toxic than entamide and the therapeutic ratio therefore, even greater

It is too early to give any detailed clinical results, but Woodruff (7), in his recent review article on amoebicides, has indicated that preliminary trials in man are very promising More clinical investigation has still to be carried out on this derivative

Discussion

Many vague syndromes and cases of alimentary neurosis have been labelled as amoebiasis, and Sir George McRobert (8) recently referred to this condition as 'chronic remunerative amoebiasis' Nevertheless true amoebic infection is still a major problem in the world It seems to us that a cheap, effective, non-

acute amoebic dysentery

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5. [illegible]
6. [illegible]
7. [illegible]
8. [illegible]

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PAROMOMYCIN AS A THERAPEUTIC SUBSTANCE FOR INTESTINAL AMOEBIASIS AND BACTERIAL ENTERITIS

KENNETH O COURTNEY PAUL E THOMPSON ROBERT HODGKINSON AND J R FITZSIMMONS

From the Research Di ision Parke Davis & Company Michigan U S A
Read by Dr B S Kaush va Central Drug Research Institute Lucknow

Paromomycin (Humatin is the Parke, Davis & Company trademark for

concise summary of the important features of paromomycin observed in the laboratory and to indicate the present status of the drug relative to the chemotherapy of certain of the enteric diseases

Laboratory Studies

Microbiologic properties in vitro *and in experimental animals* Paromomycin is highly active *in vitro* against many types of both gram-negative and gram-positive bacteria (2, 11, 13) It also proved to be very effective when tested parenterally against systemic bacterial infections in mice (1, 4, 13)

This antibiotic also has very high activity against *Entamoeba histolytica* (3)

normal bacteria

More importantly, the drug is effective against amoebae *in vivo* The antibiotic, when fed in the diet, was effective in experimentally-infected rats (3)

when administered orally, even large doses were only moderately suppressive Parenteral doses were more effective Inasmuch as the drug persists in the

in 2

It also is noteworthy that the drug has shown chemotherapeutic effect in animals against several parasites other than *E histolytica* Time does not permit reviewing the detailed observations along this line except to report that effects can be shown against all of the intestinal amœbae in rhesus monkeys, intestinal trichomonads in dogs, *Trichomonas foetus* and *Trichomonas vaginalis in vitro* and in mice and against pinworms in mice

Toxicity Paromomycin is hardly absorbed at all from the gastro intestinal tract and it is very non toxic when given orally Mice, rats, monkeys and dogs all tolerated very large amounts of the drug, either when given in one or many doses (2)

stools,

oses of

ropor

It remains to be determined whether the parenteral administration of

Clinical Trials in Intestinal Amoebiasis

in extensive trials
Over 400 reports
iave been received

Type of patients Among these patients the clinical form of the disease ranged from the chronic cyst passer through the moderately acute to the very acute type of case Further, the cases reported varied from the moderately well nourished and well nourished in those countries of the higher socio economic level to the anaemic, poorly nourished patients in the countries of a very low socio economic level

Therapy With the view to determining the minimum effective dosage required the doses of the drug were varied from 5 to 66 mg /kg /day Generally,

drug but for 3 days

Side effects Three investigators reported that beginning on the second or third day of therapy the number of daily stools was increased by two or three movements These were soft to loose but never watery in consistency There was no abdominal cramping nausea or vomiting and the bowel pattern returned to normal immediately upon the completion of therapy One patient only of the 432 cases reported had nausea and dizziness these reactions disappeared when therapy was suspended The other investigators consistently reported no adverse gastrointestinal effects and no other side reactions from the use of paro momycin

Summary of investigators reports (*Table* I) Paromomycin was used by Carter (6) in the treatment of 104 patients with chronic and subacute intestinal amoebiasis at the Sunland Training Center in Florida These patients ranged from 2 to 30 years of age and were institutionalized due to epilepsy, mental retardation or spasticity They were treated with dosages ranging from 5 to 66 mg /kg /day for 1 5 days Results were assessed within a few days after the

treated with above 12 5 mg /kg /day all were cleared The 16 patients treated with 5 mg /kg /day were not cured

Paromomycin was used in the treatment of 22 patients with subacute and protracted acute intestinal amoebiasis in Panama Treatment was for a 5 day period using 25 mg /kg of body weight per day Symptomatic improvement was noted on the second and third day and by the end of the fifth day of treat ment all patients were feeling well and their stools were negative for amoebae Follow up of these cases from 10 to 30 days after the completion of treatment revealed that the patients were still negative for amoebae

A study in Peurto Rico (7) of 20 cases of chronic and subacute intestinal amoebiasis was made using dosages ranging from 4 to 26 mg /kg /day The duration of treatment was for 5 days These patients were followed for a ninety day period at the end of which all remained free of amoebae

The antibiotic was studied in Florida in 32 children with acute and chronic intestinal amoebiasis Dosages varied from 10 to 20 mg /kg /day for a 5 day period An 11 week follow up with stool examinations on the first second third fourth sixth and eleventh weeks revealed at the end of this period only two re currences among the 32 patients

A series of 8 acute cases of intestinal amoebiasis in Honduras were treated with the drug in dosages of 22 28 mg /kg /day for a 3 4 day period Five of

these patients were completely cured, 2 required a second course of treatment [illegible]

over two months

Forty one patients with subacute to fulminating acute intestinal amoebiasis were treated in Nicaragua Treatment was for a 5 day period, 16 patients receiving 12 5 mg /kg /day, 20 patients receiving 25 mg /kg /day and 5 receiving 18 mg /kg /day In all cases symptomatic relief was obtained on the third or [illegible]

course of treatment

[illegible]

estimated 95 percent cure rate

In Ethiopia Wagner (9) treated 97 cases of intestinal amoebiasis of the [illegible]

5 day course of treatment

In South Africa, 10 cases all having ulceration of the bowel, were treated with 2 4 g daily for 10 days Sixty percent appeared to be cured during a 27-day follow up

[illegible]

and parasitologically negative

Eighteen cases of acute intestinal amoebiasis in the Philippines were treated with 25 mg /kg /day for 3 or 5 days *Of the 17 patients that returned for follow* [illegible]

amoebiasis

The Treatment of Enteric Bacterial Infections

The results of antibacterial inhibition tests *in vitro* and in experimental infections in mice have indicated that paromomycin has significant activity against groups of bacteria associated with enteric infections in man These bacteria were most notably species of *Salmonela*, *Shigella*, *Paracolobactrum* and enteropathogenic strains of *Escherichia coli* (13) Although definitive clinical trials are still in progress, the present results have shown that orally administered

TABLE I

Paromomycın (Humatın) trıals ın ıntestınal amoebıasıs (gıven orally usually 3 daıly doses)

Geograph c area	No of patients	Type of nfection	Treatment Mg /kg /day	Days	No of pat ents examined and no negative on days after treatment began				
					1 7 Exam /neg	8 14 Exam /neg	15 30 Exam /neg	31 59 Exam /neg	60 & over Exam /neg
Ch le	15	Chron c	25	5			11/11		
Egypt	20	Acute & Chron c	10-20	7 14	20/19	19/19	19/17	17 15	15/13
England	19	Chron c	30	10	18/16	18/16	1/1	6/5	4/4
Eth op a	97	Acute & Chron c	7 5 30	2 5	79/79	70/70	35/32	36/36	15/15
Honduras	8	Acute & Chron c	22 28	3 4	←		● ●		→
Mexico	26	Acute & Chron c	18 25	5	2/2	26/23	26/23		
N caragua	41	Acute & Chron c	12 5 25	5	← 4/4	31/31	★ 1/1		→
Panama	22	Acute & Chron c	25	5	←		★★		→

TABLE I (*Contd*)

Paromomycin (Humatin) trials in intestinal amoebiasis (given orally, usually 3 daily doses)

Geographic area	No of patients	Type of infection	Treatment Mg/kg/day	Days	No of patients examined and no negative on days after treatment began: 1 7 Exam/neg	8-14 Exam/neg	15 30 Exam/neg	31 59 Exam/neg	60 & over Exam/neg
Philippines	18	Acute & Chronic	30	3 5	14/13	5/5	4/4	1/1	
Puerto Rico	20	Acute & Chronic	4 26	5	20/19	20/19	14/14	10/10	10/10
U S A	32	Acute & Chronic	10 20	5	29/26	28/28	31/30	25/25	30/29
U S A	104	Acute & Chronic	5 66	1 5	36/29	31/23	60/40	19/12	21/14
Union South Africa	10	Acute	2 4 g/day	10			10/6		
Totals	432				222/207	248/234	212/179	114/104	95/85

•• Time interval for stool examinations not recorded However, investigator reported all cases Cured '
* Time interval for stool examinations not recorded for five patients However investigator reported all cases Negative
** Time interval for stool examinations not recorded However, investigator reported all cases Negative

paromomycin was effective in controlling human enteritis caused by the aforementioned bacteria Some representative examples are described in the following section

Shigellosis and infantile diarrhoea An explosive outbreak of enteritis due to *Shigella flexneri* occurred among 160 inmates of a midwestern children s Institution Approximately half of these patients were given oral paromomycin at a dose of 25 mg /kg /day and half were given 50 mg /kg day with both groups treated for 6 to 7 days Only 4 patients from each group failed to respond promptly, and stools from only 2 of these 8 patients yielded *Shigella* on culture (5)

In another study bacteriologic and clinical clearing of shigellosis occurred among 6 to 7 children given oral paromomycin at 50 mg /kg /day for 7 to 9 days An additional 17 children in the same study were treated with the same regimen of paromomycin for infantile diarrhoea caused by various enteropathogenic serotypes of *E coli* Fifteen of these 17 patients showed a prompt bacteriologic and clinical response the 2 patients who did not respond even after 14 days of paromomycin treatment were infected with the 0127 serotype (12)

Salmonellosis Paromomycin was employed for the treatment of *Salmonella typhimurium* infection among a family of 5 in Detroit after 2½ months of treatment with other drugs had failed to eradicate these bacteria from the stools Four of these 5 patients became bacteriologically negative after 5 days oral dosing with 25 mg /kg day and the other patient became negative after a second 5 day course at the same dosage All 5 patients remained well and their stools continued to be free of *Salmonella* throughout a 5-month follow up period (5)

An additional 38 patients with enteritis due to *S typhimurium* in a London hospital were treated by McMath *et al* (11) with oral paromomycin doses of 25 to 75 mg /kg /day for 5 days Most of these patients had not responded to treatment with other agents Of these 38 31 patients underwent a bacteriologic conversion subsequent to paromomycin treatment

Paromomycin was also used by Ross (12) for the treatment of salmonellosis in 9 children 7 of whom no longer had pathogens in their stools after 7 to 9 days oral dosing of 50 mg kg /day Ross also reported no side effects and no abnormalities in serial hemograms urinalyses or blood urea nitrogen and thymol turbidity tests among his paromomycin treated patients

Summary

Paromomycin is unique among known antibiotics to the extent that it is characterized by direct and marked amoebicidal action and high activity against a wide range of enteric bacteria It is well tolerated due to almost a total lack of absorption following oral administration In view of this array of properties as well as the excellent results obtained from more than 1000 patients it would appear that paromomycin will be especially useful for the treatment of amoebic and bacterial enteric infections

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Discussions

Dr K L Arora

Q Will you kindly tell us as to how a physician is to use this drug, if every case is to be judged on individual merit?

Ans A physician can best answer this question

Dr O P Tandon

Q What have been the criteria of judging the 'cure' from amoebiasis in this paper?

Ans A symptomatic phase with absence of *Entamoeba histolytica* in the stool

Dr. R M Kasliwal, Jaipur

Q This drug is supposed to have nephrotoxic properties as well as it produces diarrhoea In what way it is an advance on other similar antibiotics (like fumagillin or puromycin)?

Ans Since it is not absorbed from gastro intestinal tract there is no oral toxicity It has not been used parenterally in man Nephrotoxicity is only a point to be remembered if the drug is to be used parenterally

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NEOVIASEPT IN AMOEBIASIS

R T KAKADE

From the Hyderabad Sanatorium Hyderabad (Dn)

During the past 6½ years I had the opportunity of treating 848 cases of amoebiasis, and the following observations are based on these cases Various preparations were used for treating these cases and the results were noted comparatively As far as this paper is concerned the results pertain to neoviasept (Hoechst) tablets only

Composition

Neoviasept consist of two components (1) Bismuth salt of *p* glycolylaminophenyl arsonic acid (viasept), and (2) Chloroquine diphosphate Viasept component is effective intra intestinally while the chloroquine diphosphate is effective extra intestinally The drug is known by name Aralis in America and much work has been done there by Dr D A Barbarian and co workers and in India by Dr Sarkar Choudhary, Gupte and others

Selection of cases

All the cases selected for these trials had either *Entamoeba histolytica* vegetative forms or cysts present in the stools Though some of these cases never presented themselves with cardinal symptoms of amoebiasis they were found to be suffering from this disease

The total number of cases treated only with neoviasept was 216 of which 106 were males, 61 females and 49 children

Patients of all ages and both the sexes were found to be suffering equally with this disease No age seems to be exempt My youngest patient was 8 months old and the oldest was 76 years of age

Distribution of cases

1	*Alopacia aereata*		3
2	Cardiac cases		8
3	Ear (Meniere's disease)		1
4	Eye		1
5	Fits		6
6	Hepatic involvement		16
7	Intestinal	(*a*) acute	16
		(*b*) chronic	93
8	Lungs	(*a*) pulmonary	2
		(*b*) asthamatic	21
		(*c*) eosinophillic	11
		(*d*) empyema	2
9	Neuritis and neuralgic		26
10	Skin	(*a*) eczema	4
		(*b*) vitiligo	4
11	Spongy gums		2
		TOTAL	216

(i) Alopacia aereata

Three patients presented with the main feature of *Alopacia aereata* patches quite resistant to all other treatment On examination of their stools *E H* cysts were found and so these cases were treated with neoviasept with full success Falling of the hair is a common symptom in the amoebiasis patients

(ii) *Cardiac cases*

These patients come with tachycardia, anginal pains, precordial pain, extra-systole, palpitation, low blood pressure, fainting attacks etc

(iii) *Meniere's disease*

A case diagnosed as Meniere's disease was referred to me On a casual examination of the stools *E H* cysts were found The patient responded very well to anti-amoebic treatment with neoviasept and vitamin B-complex therapy

(iv) *Eye*

A case of Iridocyclitis was referred for a check up by the opthalmologist as the patient had *E H* cysts in his stools The ocular lesions improved with neoviasept treatment

(v) *Fits*

Some cases of idiopathic fits were examined and found positive for *E H* cysts These cases were relieved of their fits with antiamoebic treatment

(vi) *Hepatic involvement*

It is the commonest complication of amoebiasis Some patients had severe pain in abdomen and in right shoulder joint tip with high fever and leucocytosis Some had developed the hepatic abscess and some abscesses burst into thoracic cavity giving rise to empyema I could get the best results in these hepatic cases with neoviasept tablets

(vii *a*) *Acute intestinal amoebiasis*

The diagnosis in these cases is quite easy Vegetative forms are generally found in these cases These cases responded very well to neoviasept in conjunction with emetine hydrochloride and vitamin B-complex

(vii *b*) *Chronic intestinal amoebiasis*

The symptoms vary from simple heaviness and dull ache in the abdomen to acute abdominal catastrophe simulating acute appendicitis, perforating gastric ulcer, biliary colic, renal colic, etc Loss of appetite, lassitude, forgetfulness, loss of concentration, feeling of uneasiness, growling abdomen, constipation, dyspepsia etc , are the commonest symptoms Onset in many a cases is insidious and patient is unaware of the infection he is harbouring Only 38% of the cases come with abdominal symptoms

(viii) *Lungs*

(a) *Asthamatic attacks*—Some patients come with paroxysmal attacks of cough as their main symptom *Breathlessness, wheezing, difficult breathing,* cynosis make the picture simulate '*Status Asthamaticus*' Perhaps this is an [illegible] to foreign protein produced by the presence of *E H*

[illegible] initis These cases cleared

[illegible] i e , *E H* cysts was era-

[illegible] ilic count ranging from 8

[illegible] ntiamoebic treatment with

[illegible] l eosinophilia were found

to be positive for *E H* Sometimes it takes months to find *E H* cysts in the stools of such patients One very interesting finding I came across was that,

[illegible]

[illegible] test was negative in both these cases and later *E H* cysts were found in stools Patients were treated on the anti amoebic line and the X rays taken six months later were quite clear In some cases where the infection has reached the liver give rise to the right basal infiltration due perhaps to the upward rise of the liver and congestion of right base which is many a times wrongly diagnosed as the cases of right basal lesion of tuberculosis

Two cases of amoebic empyema on the right side were treated by aspiration and antiamoebic line of treatment with neoviasept and emetine with very good results

(ix) *Neurasthenia, neuralgia and neuritis*

This group of cases was present in a large number Patients generally come with these as the main symptoms and do not have any other trouble Any nerve in the body may be affected such as trigeminal facial, sciatic or some small peripheral nerve I have now made it a rule to examine the stool of every such patient as a routine These symptoms are probably due to the toxin produced by the cysts in the body Tobacco in any form aggravates these nervous symptoms and better results are achieved by asking the patients to stop smoking or chewing tobacco While treating these cases satisfactory results are achieved if the therapy is combined with massive doses of vitamin B

(x) *Skin*

The commonest and allergic phenomenon seen is eczema Eczema with amoebiasis responds very well to neoviasept course with vitamin C Another very interesting finding is vitiligo with raised border

(xi) *Spongy gums*

Chronic amoebic cases have yet another common symptom *i e*, spongy and bleeding gums Very resistant cases which did not respond to massive doses of vitamin C, calcium, vitamin A and K but responded very nicely to anti amoebic treatment with neoviasept

Dosage

1 *Male* For male adults two tablets three times a day for 12 days was quite effective though some of my colleagues advocated six tablets a day for 8 days

2 *Female* Four tablets a day for 12 days

3 *Child* Three tablets a day for 12 days

As already mentioned above better results are achieved if vitamin B is also given in conjunction with neoviasept

After the 12 days course a gap of 2 weeks is given and stools are examined for *E H* cysts If the stools are found positive the same course of 12 days is repeated The stools are repeatedly examined for the next six months to two years at the interval of a fortnight and if still the patient be found positive, he was given the third course of neoviasept for another 12 days The very resistant cases required the third course

The following table will show the results of the cases and the number of courses given to them

TABLE I

Table showing number of courses given for complete cure

Sex	1st course	2nd course	3rd course
Males	106	16	4
Females	61	9	3
Children	49	11	6
Total	216	36	13

Follow-up

Out of 216 cases, 138 cases could be followed for the following time

Cases followed for 18 months	74
Cases followed for 2 years	36
Cases followed for 3 years	16
Cases followed for 4 years	8
Cases followed for 5 years	4

[illegible] How- [illegible] l com- [illegible] te was the minimum and the percentage of cure is the highest Not a single case was found to be positive after giving the third course of neoviasept

Side-effects and after effects

Side-effects and after-effects were practically negligible Some of the patients complained of nausea, loss of appetite, mild degree of pain in abdomen, headache and very rarely vomiting, if neoviasept tablets were given alone When given in conjunction with vitamin B complex, no patient ever complained of any side-effects

The follow-up shows no toxic effect of the drug even after giving the three courses to the patients

Conclusion

The trials conducted with neoviasept, other anti amoebic drugs and antibiotic drugs for amoebiasis show that the best results are achieved with neoviasept in acute as well as in chronic amoebiasis, may it be intestinal or extra-intestinal Acute cases respond very well if the therapy is combined with emetine and vitamin B-complex Chronic cases (intestinal and extra-intestinal) respond very well with neoviasept combined with vitamin B About 16% required course to be repeated twice and 5% required course to be repeated thrice No person required more than three courses when treated with neoviasept

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LNIOBEX IN AMOEBIASIS

P K GHOSH AND S GUPTA
From the Mayo Hospital Calcutta

(ı l ı ı \ ı ı

examined in a general hospital contained *E histolytica* infection—or one in every 5 stools

In a similar bigger hospital in a semi urban area (Burnpur) among 4 211

hosp tals much more male patients attend than female pat ents

The age distribution among our 85 cases are as follows

0—10 yr	20
11—20	16
21—30	28
31—40	18
41—50	1
51 & above	2

Thus the infection is almost equally prevalent in all ages up to 40 yr in urban area Infants and children are no exception they constitute 23 5% or nearly quarter of the cases in the series There are 2 cases aged 1 yr and 8 cases upto the age of 5 yr Seventyfive percent of the cases were in persons up to the age of 30 yr

The presence of other bowel infections It is difficult to prove that *E hystolyti* favours other infections by lowering local bowel resistance—but other protozo and helminthic infections are associated The latter are rather concomitai than result of *E histolytica* infection In urban areas the association with giardi is remarkable—94 cases (29%) in 322 amoebic stools In semi-urban and rur areas giardia is less common—10 cases of helminthic infection only among 12 amoebic stools (nearly 8%) Whether *E histolytica* has harmful effect on norm bacterial flora engaged in synthesis of vitamin B complex in the intestines in th same way, as has been noticed after the use of some antibiotics, remains yet t be worked out Possibly it does, for the dyspeptic symptoms and bowel disorde are akin to symptoms of B-complex deficiency

Entoblx

L histolytica causes acute and chronic bowel disorders Emetine i beneficial in acute cases Chronic cases are difficult to cure and continue t recur Many preparations are used in their treatment, *e g*, *Kurchi*, vioform

In *in vivo* (rat) marked amoebicidal action is noticed with 50-75 mg/kg by mouth for 5 days In larger doses it produces vomiting and minute hematuri in animals (dogs) In 600 mg daily dose in man it induces vomiting Therapeutically

plus
minary stool examination—no case was included where EH, cysts or vegetative forms were not found The cases were all ambulatory except five The dose given in adults was one tablet 3 times a day for 10 days, or 2 tablets 3 times a day for 3 or 4 days, then 1 tablet 3 times a day for 6 or 7 days Children received [illegible] The stools became cyst-free in 7-10 days on an) with 6 tablets a day Vegetative r schemata Stools were examined the institution of treatment—and upto 4 weeks in some instances

There were 52 male and 11 female cases The lowest age was 1 yr and highest 71 yr Cysts were present in 35 (55 5%), vegetative form in 20 (31 7%) and both forms in 8 cases (12 8%) In 6 samples giardia cysts, and in another 8 samples ova of hookworm and/or roundworm were also found

The total number of days of treatment was 583 days for 63 cases, or 9 2 days per case Twentyeight cases (44%) were treated for 10 days, 27 (43%) for 11 18 days, and 8 (13%) for 4-6 days

Results

Clinical relief (cure) was obtained in all 63 cases The cysts persisted n stools in 3 cases—in all others the cysts had disappeared Giardia cysts

numbers,
of giardia from the stool But it had no effect on ova of hook worms Enlarged and/or tender liver also improved and became smaller

There were 3 cases of mild nausea and one case of allergic dermatitis (scalp) in this series Eleven cases suffered from constipation There were no other toxic manifestations

In the second series entobex alone in 50 mg doses was tried in 25 cases Three cases did not complete the course of treatment In the final analysis there were [illegible]
1 and 55 yr [illegible]
yr range [illegible]
continued f [illegible]
usually 7, 10 and 15 days after commencement of treatment Results of treatment was considered good in 13 cases (59%) there was clinical cure and the cysts [illegible] In 7 cases (32% [illegible] 2 cases (9% the re [illegible] —cysts dis appea [illegible] amination
Seven cases [illegible] complained of constipation There were no other toxic manifestations

CONCLUSION

[illegible]

above toxic manifestations were absent It is thus both a safe and potent drug Besides its anti amoebicidal action it seems to be active also on giardial infection

The presence of other bowel infections It is difficult to prove that *E histolytica* favours other infections by lowering local bowel resistance—but other protozoal and helminthic infections are associated The latter are rather concomitant than result of *E histolytica infection* In urban areas the association with giardia is remarkable—94 cases (29%) in 322 amoebic stools In semi urban and rural
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Entobex

E histolytica causes acute and chronic bowel disorders Emetine is beneficial in acute cases *Chronic cases are difficult to cure and continue to* recur Many preparations are used in their treatment, *e g*, *Kurchi*, vioform,

mouth for 5 days In larger doses it produces vomiting and minute hematuria in animals (dogs) In 600 mg daily dose in man it induces vomiting Therapeutically 300 400 mg is a safe dose

In one series of 63 cases of intestinal amoebiasis, the effect of entobex (20 mg) plus enterovioform (200 mg) was studied Every case was selected after a preliminary stool examination—no case was included where EH, cysts or vegetative forms were not found The cases were all ambulatory except five The dose given in adults was one tablet 3 times a day for 10 days, or 2 tablets 3 times a day for 3 or 4 days, then 1 tablet 3 times a day for 6 or 7 days Children received correspondingly lower doses The stools became cyst free in 7-10 days on an average with 3 tablets a day—earlier (4 5 days) with 6 tablets a day Vegetative *forms also disappeared in 3 5 days with the latter schemata* Stools were examined 4 or 5 times in each case upto 3 weeks from the institution of treatment—and upto 4 weeks in some instances

There were 52 male and 11 female cases The lowest age was 1 yr and highest 71 yr Cysts were present in 35 (55 5%), vegetative form in 20 (31 7%) and both forms in 8 cases (12 8%) In 6 samples giardia cysts, and in another 8 samples ova of hookworm and/or roundworm were also found

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There were 3 cases of mild nausea, and one case of allergic dermatitis (scalp) in this series Eleven cases suffered from constipation There were no other toxic manifestations

In the second series entobex alone in 50 mg doses was tried in 25 cases Three cases did not complete the course of treatment In the final analysis there were thus only 22 cases (13 male and 9 female) The ages varied between 1 and 55 yr (7 in 0 10 yr, 9 in 11 20 yr, 3 in 21 30 yr, 2 in 31 40yr, and 1 in 55 yr range The dose was either 1 tablet 3 times daily or 2 tablets 3 times daily, and continued for 10 days Besides the preliminary examination stool was examined usually 7, 10 and 15 days after commencement of treatment Results of treatment was considered good in 13 cases (59%) there was clinical cure and the cysts were negative on two examinations on the 10th and 15th day In 7 cases (32%) there was clinical improvement, but the cysts persisted In 2 cases (9% the result was considered doubtful—there was clinical improvement—cysts disappeared on second examination, but reappeared on the third examination Seven cases (33%) complained of constipation There were no other toxic manifestations

Conclusion

Entobex is a potent anti amoebicidal drug In 59% of cases it cured the disease and caused disappearance of cysts from the stool In combination with enterovioform its action seems to be further enhanced Thus in 63 cases where this combination was tried, 60 cases responded favourably In the doses given above, toxic manifestations were absent It is thus both a safe and potent drug Besides its anti amoebicidal action it seems to be active also on giardial infection

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TREATMENT OF AMOEBIASIS

A CLINICAL TRIAL OF NEW SYNTHETIC COMPOUND—11925-C*

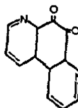
J G PAREKH AND BABUBHAI D PATEL

From the J J Group of Hospital, Bombay

Amoebiasis is common in our country The last word in its treatment has yet to be said. Fairly large number of drugs are available, each one with a variable effect on one or the other stage of the parasite Most of these drugs cause disappearance of the symptoms and of the parasites, at least temporarily Relapses and reinfections, however, are common but it is difficult to differentiate them or prevent them and the search for better drugs continues

A new synthetic compound 11925-C, 4 7-phenanthroline-5 6-quinone,

[illegible]



I

Microbial interrelationships are involved in the aetiology, pathogenesis and pathology of intestinal amoebiasis By itself in absence of bacterial flora, *E histolytica* appears not only incapable of producing the disease but it does not even survive for more than a few days A synergism of amoeba and bacteria is a pre-requisite to the development of the disease(2)

Since 11925-C is claimed to possess a dual action both anti-amoebic and antibacterial it could be expected to prove to be a better drug than the existing amoebicidal drugs

It is slightly soluble in water and it is rapidly absorbed It is excreted in bile and urine (1) It is relatively free from toxic effects in animals In men, except for occasional nausea and vomiting, and some minor toxic effects, *e g*, headache diarrhoea, petechial rash, etc, no serious toxic effects have been reported (3—6)

The compound, 11925-C, has been under clinical trial all over the world for over 2 years and several reports have appeared in this country (3, 7—10)

This paper deals with a clinical trial of this new phenanthroline derivative in amoebiasis

* Entobex (CIBA)

Material and Methods

A series of 135 adult patients attending J J Hospital (out patients department) were taken up for the present study, on finding the vegetative and/or cystic forms of *E histolytica* in the stool examinatin

A detailed history of the complaints and past history of diarrhoea, dysentery or gastro enterological disorders were recorded The patient's physical examination was carried out in details The stools were examined usually on alternate days during the first 10 days of the treatment and then at the end of a fortnight, a month, and 2 months in as many cases as possible Sigmoidoscopic examination was carried out before and after the treatment in a very small number of cases At the same time clinical progress was noted

After therapy, the stools were examined with few exceptions by merthiolate iodine formaldehyde concentration method (Dave), to ensure greater accuracy in assessing the results

For the purpose of treatment, the patients were divided in three groups [illegible] ht or one [illegible] bination [illegible] of the [illegible] times a [illegible] 1 5 *i e*, [illegible] h group received the respective treatment for a period of ten days No other anti amoebic treatment was given

Findings

[illegible] The common symptoms were pain in abdomen (71%) and [illegible]

TABLE I

Clinical manifastations

	Total	Percentage
Total cases	135	
Acute dysentery	17	13
Chron c dysentery	90	66
Asymptomatic cases	12	9
Pain in abdomen	96	71
Abnormal bowel movements	127	93
Frequency of stools	33	24
Irregular bowel habits	52	38
Constipation	42	31
Previous history of diarrhoea or dysentery	79	58
Enlarged tender liver	2	

Mexaform (CIBA)

The results of microscopic examination of the faeces in the 135 cases are presented in Table II

TABLE II

Findings on examination of faeces

	Total	Percentage
Total number of cases	135	—
Cyst of *E histolytica*	118	82
Vegetative *E histolytica*	7	5
Vegetative & cystic *E histolytica*	10	7
More than one parasite	76	56
Cyst of *E coli*	33	24
Cyst of *G lamblia*	21	14
Cyst of *I butchli*	3	2
Ova of ascaris	21	14
Ova of trichuris	22	14
Ova of ankylostome	5	3 5

Results of Treatment

The criteria adopted for the successful therapy were the clinical improve-

cases

TABLE III

Results of therapy

	Group I	Group II	Group III	Total
Number of cases	38	57	40	135
Cases completely studied	28	36	32	96
Immediate cure	25	29	32	86
Percentage immediate parasite clearance	89	80	100	88
Relapses	3	4	5	12 5%
Percentage of final parasite clearance	78	69	84	77

Of the 135 cases 96 could be adequately followed Eightysix (88%) had immediate parasite clearance Seventyfour (77%) had both—symptomatic relief as well as parasite clearance at the end of the follow-up Acute manifestations responded better than chronic manifestations *E coli* and *G lamblia* were also favourably affected

Groupwise, the group III in which the combination of 11925 C (50 mg) with enterovioform (250 mg) 3 times a day was used, gave best results having 100% parasite clearance at the end of ten days' therapy While 27 cases, i e, 84% were cured as observed during the follow-up

In group I, treated with 11925-C alone, out of 28 cases, 25 (89%) were free of

E histolytica at the end of 10 days while 22 cases (78%) were cured in the later follow up

In group II, of the 36 cases, 29 (80%) did not pass *E histolytica* at the end of therapy, while 25 (69%) were completely cured as seen by the later follow up

Discussion

[illegible]

bowel movements, chronic dyspepsia were other common features Two patients had enlarged tender liver

Multiple parasitic infestation was a common feature Some cases had as many as 4 to 6 varieties of parasites (both protozoa and helminths) As many as 56% had more than one parasite Most common accompaniment was *E coli* (24%)

In view of the lack of complete satisfaction regarding the treatment of amoebiasis, attempts have been made to produce newer synthetic drugs to bring about better results Compound 11925 C is one such drug Out of 135 cases taken up for this study, 96 could be adequately followed for a period of two months The results of our study indicate that 11925 C produces immediate [illegible] at the end of ten days It also influences *G lamblia* [illegible] ias given slightly better results [illegible] combined with 750 mg of enterovioform, the results were still better but not statistically significant There were no serious side effects in any of the patient, except two cases who had abdominal pain during the therapy

Sagone (5) treated 720 patients of amoebiasis with 11925 C for 8 days and repeated the course after 2 3 months He obtained stool clearance in 92 5% [illegible] of 8 days He used on an average 30 mg daily In [illegible] Nagaty, *et al* (11) [illegible] estinal amoebiasis in [illegible] 12 mg per kg body [illegible] were obtained when larger doses were [illegible] achdev (6) [illegible] r kg body [illegible] 7) observed [illegible] In Sagon's [illegible] e still stool [illegible] ol clearance [illegible] ver, 30 1% were stool positive after three months The author, nevertheless, considers 11925 C to be more effective than emetine, well tolerated and a better amoebicide than many now in use He also found it encouraging in the treatment of other intestinal protozoal infections

Its action on amoebic hepatitis is controversial as reported by some authors (4 8—10 12)

[illegible]

the fact that mere stool [illegible] therapy could not be used as a gauge of the value of the drug, for eradication of

the infection Any contact amoebicide may render the stool negative *temporarily* as mentioned by Adams (3)

Summary and Conclusions

This study presents the results of a clinical trial given to a new synthetic phenanthroline quinone derivative in 135 cases of amoebiasis 96 cases could be studied adequately

The cases were divided in three groups The treatment was given for ten days In group I, the patients were given 50 mg of compound 11925 C three times a day

Out of 28 cases, 25 (89%) were amoeba free at the end of therapy and 22 cases (78%) were free at the end of the follow up

In group II, the patients were put on 20 mg compound 11925 C+200 mg enterovioform three times a day

Of the 36 cases 29 (80%) were amoeba-free at the end of therapy while 25 (69%) were free at the end of follow up

In group III, patients were given 50 mg, 11925-C and 250 mg of enterovioform three times a day

Out of 32 cases studied, all (100%) cases were amoeba-free at the end of ther py but 5 cases again showed the parasites during the follow-up, thus 27 (84%) were free

Thus the best results were obtained in group III with compound 11925-C giving in the dose of 50 mg along with enterovioform 250 mg, both three times a day, for ten days

Two cases of amoebic hepatitis patients in group II did not respond satisfactorily

The incidence of *E coli* and *G lamblia* was also reduced after the course of treatment

The drug was found to be well tolerated, non-toxic and with high therapeutic index

The clinical and amoebicidal effects compare favourably with the currently used drugs

Acknowledgments

Our thanks are due to the Professor of Pathology, Dr R K Gadgil, the

to carry out this work and publish this paper

We are thankful to Dr B L Silveira, Manager, Clinical Research Division, M/s CIBA Pharma Private Ltd, Bombay for supply of the drugs used in this trial

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CLINICAL TRIAL WITH PHENANTHROLINE COMPOUNDS IN THE TREATMENT OF CHRONIC AMOEBIASIS

P K CHATTERJEE

Calcutta

Two phenanthroline compounds namely, 4 7-phenanthroline, 5 6 quinone (Entobex or 11925C) and its semicarbazone (11925) have now been given a fair trial by a number of Indian workers, Sen *et al* (1), Sen and Sanyal (2), Roy Chowdhury *et al* (3), Chatterjee *et al* (4), Das (5), and Sachdev and Daupar (6) Both the compounds were found to have strong amoebicidal action *in vitro* as well as bactericidal action, entobex being twice as strong as its semicarbazone derivative (personal communication from the scientific department of CIBA Pharma) The amoebicidal action of the compounds *in vivo* was also studied in experimental amoebiasis of rats and was found satisfactory In animal experiments the compounds were found to be well tolerated

Material

Since 1957 a total of 127 patients have been treated with the compounds. Of these 9 were treated for infections other than amoebiasis, 22 patients did not complete the course and in 4 patients treatment had to be abandoned due to severe intolerance Excluding these 35 cases, a total of 92 patients considered to be suffering from chronic amoebiasis were treated with a course of these compounds

Patients with chronic or recurrent diarrhoea with mucus in stool, vague discomfort or pain in abdomen, palpable and tender caecum, showing *E histolytica* in their stool, in cystic or vegetative forms, either alone or with giardia, were selected for treatment A few cases were also included in the trial, although a single examination of their stool was negative, because they showed the above symptom complex and no other obvious cause for the symptoms could be found These were considered as clinically diagnosed cases and their results were considered separately Patients with other vague abdominal symptoms such as anorexia flatulence, irregular bowels, etc , were also selected for treatment if their stool examination showed presence of *E histolytica* and no other obvious cause for their symptoms could be found

Sixtynine cases had positive stool (group I) and 23 cases were clinically diagnosed (group II) Most of the patients were between 20 and 40 years of age About half the patients were attending the out-patient department during treatment and the rest were in hospital at bed rest

Treatment

The compounds were given in tablet form by mouth One group was treated with 11925 (semicarbazone derivative) (100 to 200 mg) 3 times a day, another group was treated with entobex (11925C) (100 mg) 3 times a day, a third

group was treated with a combination of entobex and enterovioform In this last group four different dosage schemes were followed

	Entobex	Enterovioform
Scheme I	50 mg twice daily	250 mg thrice daily
Scheme II	50 mg thrice daily	,, ,, ,,
Scheme III	40 mg ,, ,,	400 mg ,, ,,
Scheme IV	20 mg ,, ,	200 mg , ,,

RESULTS

In assessing

vement in charac

taken into accour

stools were positive or not

Group I (with positive stool) 69 cases

Group II (clinical cases) 23 ,

In group I 25 cases were treated with 11925 (semicarbazone derivative) and the clinical improvement was seen in all of them One case had acute dysenteric symptoms which were controlled in 5 days Chronic loose stools with mucus were present in 13 cases In all the cases stools improved in charac r and number in 3 to 6 days At the end of 10 days treatment *E histolytica* disappeared from stools in 23 of the 25 patients In 3 patients liver was enlarged and tender before treatment In all the 3 cases there was marked improvement after 10 days treatment In 2 cases although some clinical improvement occured stools were still positive after 10 days' treatment Most of the cases were treated

dose had to be reduced

In the 2 cases where

a day was given

Eleven cases of group I were treated with entobex One of them had acute dysenteric symptoms and four had chronic loose stools In all these 5 cases diarrhoea was controlled and stools improved in character in 3 to 6 days In

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of 100 mg 3 times a day In those cases who failed to respond an increase of

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continued In 4 cases originally selected for treatment with 11925 treatment had to be abandoned due to serious symptoms of intolerance With entobex symptoms of intolerance were much milder and treatment could be continued in all cases

In view of these facts it was decided to try entobex the better tolerated

Twentyfive cases of group I showed marked improvement in clinical signs and symptoms as well as in character of stool In 19 of these cases stools were examined after completion of treatment and were found negative in 15 In 4 cases stools were still positive after treatment In Group II, 17 of the 23 cases showed marked improvement

As mentioned before four different dosage schemes were followed in the combined treatment The results under these schemes are shown separately in Table I

Group I—Cases with positive stool

Group II—Cases diagnosed clinically

On the individual symptoms in the two groups acute dysenteric symptoms were relieved promptly in 9 out of 13 cases and chronic loose stools were controlled in 17 of 25 cases Enlarged and tender liver was present in 13 cases In one case only marked improvement was seen and in 6 cases only slight improvement occurred

TABLE I

	Group I			Group II		
Dosage scheme	Total treated	Improved	Not improved	Total treated	Improved	Not improved
I	5	5	0	4	2	2
II	3	3	0	1	1	0
III	13	11	2	9	8	1
IV	12	6	6	9	6	3
Total	33	25	8	23	17	6

In the combined treatment the dose of entobex varied from 150 mg to 60 mg per day From Table I it will be seen that all the cases treated under schemes I and II showed marked improvement in group I But in scheme IV with 60 mg of entobex and 600 mg of enterovioform per day the results were less satisfactory than with scheme III with double the dose of the two drugs

Tolerance Both the compounds were well tolerated Only in higher doses symptoms of intolerance mainly gastro intestinal irritation were seen There seemed to be considerable individual variation in tolerance While some patients could tolerate even 600 mg of 11925 and 11925C a day, others had trouble with even 150 mg a day only The symptoms of intolerance seen were anorexia nausea vomiting dryness of mouth diarrhoea and burning in the eyes Roughly, one third of the patients showed some symptoms of intolerance The entobex was better tolerated and symptoms of intolerance were mild Of all the cases originally selected for treatment intolerance was severe enough to abandon treatment in 4 cases only All these cases were treated with the compound 11925

Discussion

Results of treatment of chronic amoebiasis are notoriously difficult to assess While immediate relief of symptoms and disappearance of parasites from stools may occur with many drugs relapse rate is high In a short term trial therefore [illegible]

[illegible]

involving absence from work Most physicians in general practice, therefore,

prefer a course of treatment which is easy to follow at home with the least possible disturbance of the patients' rout ne of work, specially in cases who are not ill enough to take to bed In hospital practice also a readily effective and safe oral therapy suitable for out-patient treatment of the large number of chronic amoebiasis patients would be very useful

It is generally recognised that for effective control of amoebic infection better results are obtained by combining an amoebicidal drug with anti bacterial drugs The two compounds under trial have both strong amoebicidal as well as bactericidal properties Moreover, they are absorbable so that they may

In view of these facts

satisfactory With the compounds 11925 (semicarbazone derivative) marked clinical improvement was seen in all the 25 cases treated Acute and chronic diarrhoea and dysenteric symptoms were quickly controlled Parasites disappeared from stool in 23 of 25 cases With the Compound 11925C (entobex), when used alone, 6 of the 11 *cases showed marked clinical improvement with disappearance of amoebae* from stools Here also diarrhoea and dysenteric symptoms were quickly controlled The number of cases treated with entobex alone, however, was small But 33 other cases with positive stool were treated with a combination of entobex and enterovioform, and marked clinical improvements was seen in 25 of these cases In another group of 23 cases diagnosed clinically, the combined treatment was given and marked improvement was seen in 17 of these cases

It was further seen that with both the drugs and in combined treatment, cases with more acute symptoms showed better results Trophic forms of *E histolytica* disappeared more frequently than cystic form It may thus be concluded that both the compounds are very useful for immediate symptomatic relief of chronic amoebiasis In the so-called chronic cyst-passers with mild or no symptoms, the results are less satisfactory

Although both the compounds were quite effective in dosage of 300 mg a day, symptoms of intolerance were seen in almost one third of the cases As entobex was better tolerated and was found to be twice more effective *in vitro*, it was decided to try entobex in smaller doses in combination with enterovioform It was seen that in this combination 100 to 150 mg of entobex a day was quite well tolerated and the therapeutic results were satisfactory It would seem *therefore that combination of a locally effective amoebicidal drug like* enterovioform with an absorbable amoebicidal and bacteriostatic drug entobex would provide a very useful therapeutic weapon for outdoor treatment of chronic amoebiasis

Acknowledgment

I am grateful to Dr M N Sarkar, Superintendent of the Calcutta National Medical College Hospital, for his kind permission to carry out the trial and to publish the results, and to Messrs CIBA Pharma (Private) Limited for a generous supply of the compounds 11925, 11925C and enterovioform

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PRELIMINARY EXPERIMENTAL AND CLINICAL RESULTS WITH A NEW SYNTHETIC EMETINE-LIKE COMPOUND

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From the Hoffmann La Roche & Co Ltd Ba.le Sut.erland

CHEMISTRY

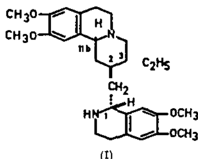
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In the *emetine series* various stereoisomers were synthesized Antiamoebic activity was found only in the 2 3 *trans* series, which corresponds in this respect to the stereochemistry of the natural alkaloid (I)

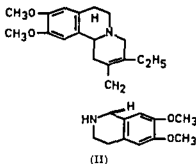


(I)

However, of the racemic isomers obtained, only one, the racemic emetine itself, showed the expected antiamoebic activity (4)

The other racemic *iso* emetine is much less active Racemic emetine could then be separated into the two optical isomers and it was observed that only the (—) form, identical in every respect with the natural product, was active but not the unnatural (+)-form

The synthesis of 2 dehydro-emetine series, a new group of compounds, was achieved by Brossi *et al* (3, 5) This synthesis is much easier than that of the emetine analogues just mentioned, due to the presence of a double bond in their ring system which reduces the number of asymmetric centres from 4 to 2 and consequently the number of racemates from 8 to 2 (II)



(II)

Since the 2 dehydro emetine series conserves all the antiamoebic activity of the natural series, we concentrated our work on it Here again, the synthesis leads in the last step to two racemic stereoisomers Only one, which probably corresponds in its two asymmetric centres to the stereochemistry of emetine, showed marked antiamoebic properties *The second, racemic 2 dehydro-iso-emetine*, was inactive The synthesis of other 2 dehydro emetine derivatives with different substituents in the molecule has not led to any further interesting products Racemic 2 dehydro emetine (Ro 1 9334) itself was therefore chosen to start clinical trials

TOXICOLOGY

Acute toxicity

The lethal doses of Ro 1 9334 and emetine after a single administration by different routes was studied on the mouse The figures obtained are shown in Table I

TABLE I

LD mouse	Emetine			Ro 1 9334		
	oral	s c	i v	oral	s c	i v
10%	28	28	14	35	58	24
50%	35	35	18	50	70	28
90%	43	43	22	70	88	35

Emetine thus seems to be almost twice as toxic as Ro 1 9334

Subacute toxicity

normal but both preparations produced a slight neutropenia with relative lymphocytosis

The histologic studies of the organs of animals receiving Ro 1 9334 revealed only a slight turbid tumefaction of the liver and a diminution of lymphatic tissue in the spleen

Chronic toxicity

contained in Table II

TABLE II

Amount contained in feed (%)	Ro 1 9334		Emetine	
	Amount absorbed daily (mg /kg)	Effects	Amount absorbed daily (mg /kg	Effects
0 05	11 2	All animals dead after 1 week	30*	All animals dead after 1 week
0 01	4 5	All animals dead after 2½ weeks	6*	All animals dead after 2 weeks
0 005	3 5	All animals survived 12 weeks	3	All animals dead after 2 weeks

*Dose estimated theoretically

nd emetine even at the lowest daily weeks are still highly toxic How ived while those receiving emetine is better tolerated Furthermore,

the animals receiving Ro 1-9334 showed a weight loss of ⅓ to ½, slight turbid tumefaction of the liver and myocardium, but no alteration in other organs or the blood count

Pharmacology

Intravenous administration of the compound Ro 1-9334 in doses of 2, 5 to 5 mg /kg produces transient hypotension in the cat anesthetized with Numal In doses of 5 and 10 mg /kg it provokes a prolonged decrease in the *blood flow* of the carotid and femoral arteries These effects are less intensive than with emetine

In doses of 10 mg /kg Ro 1 9334, like emetine, reduces the *pulse rate* by 12% during 10 to 15 min

The effect of Ro 1-9334 and emetine on the electrocardiogram was studied on rats by intravenous infusion at constant volume and speed of differently concentrated solutions In these acute toxicological trials, where 1 8 to 0 18 mg /kg per minute were injected, appeared in both substances marked electrographic changes consisting in abnormal ventricular complexes, a-v lengthening, extrasystoles, extreme bradycardia However, in order to provoke death by cardiac arrest in 5-10 min about the double doses of Ro 1 9334 compared with emetine were necessary Both the substances in 0 18 mg kg per min could be injected during 120 min without altering the basic pattern of the electrocardiogram

The *respiratory volume* increases by 20% after i v administration of 5 mg /kg and by 80% after doses of 10 mg /kg These effects last 3 and 2 min respectively, and are followed by a reactional decrease during 8 min Emetine has a rather depressive action in similar conditions In concentrations of 2×10^{-5} and 2×10^{-4}, Ro 1 9334 relaxes the *spasmodic effect* of $BaCl_2$ and in a concentration of 2×10^{-4} also that of acetylcholine The same concentration of the compound stimulates the isolated uterus of the rabbit and reduces the action of adrenaline The effects do not seem to be specific

The *emetic effect* of Ro 1-9334 and emetine was studied on the dog after [illegible] observed after 1 mg /kg
[illegible] e produced 56 retching
[illegible] the same oral dose of
[illegible] after 45 to 170 min
[illegible] as well as subcutane-

Experimental Chemotherapy

Antiamoebiasic activity

[illegible] the concen-
[illegible] and the oral
[illegible] ther It can

TABLE III

Drug	LD_{50} mouse mg /kg oral	s c	*In vitro* γ/cc end point	*In vivo* CD rats mg /kg
Ro 1 9334	50	70	1 100	1 25—3 85
(—) Emetine, synthetic comp equivalent to natural emetine	35	35	1 100	3 1 —7 8
(+) Emetine, synth comp corresp to the opt isomer of nat emetine	—	700	1000	170

be seen that Ro 1-9334 and (—) emetine the natural alkaloid, have similar toxicity and *in vitro* activity, but that the *in vivo* activity of Ro 1 9334 is about twice that of emetine (+) Emetine is atoxic but inactive

Action against Schistosomiasis mansoni *of the mouse*

Daily treatment of mice with 10 mg kg of Ro 1-9334 for a fortnight beginning 32 days after infection with Cercaria of *Schistosoma mansoni* provokes a significant reduction in the number of worms in the liver and in the mesenteric and portal veins The effect is almost the same whether administration is oral, subcutaneous or intraperitoneal

Preliminary Clinical Results

The clinical trial of Ro 1 9334 began a year ago in a limited number of medical centres The experience gained does not yet allow definitive conclusions However, some provisional statements can already by made

Amoebiasis

About 40 cases of acute hepatitis and hepatic abscess, and 20 cases of acute intestinal amoebiasis have been treated so far with subcutaneous or intramuscular injections of the 10% solution of Ro 1 9334 The drug has shown a distinct therapeutic effect and the cure rate obtained seems to be of the same order as the one of emetine

As these cases were the first patients treated and it was not known how well the drug would be tolerated, the dosage employed was rather low and not uniform (30 80 mg daily over 10 days) This could explain the few cases which did not respond as promptly as they might have done to emetine

Schistosomiasis

Ro 1 9334 has shown an evident effect on about 60 patients with *Schistosomiasis mansoni* (6 7) The parasitological cure rate appears to be 40 60% with the rather low dosage so far employed Results will probably improve with the higher doses now used (80-120 mg daily over 10 days) Trials have also started on *S haematobium* and *S Japonicum*

Tolerance

Systemic side effects were infrequent after doses of 0 5—1 0 mg /kg over 10 days and somewhat more frequent but never alarming, after 1—2 mg /kg Thus Ro 1 9334 seems to be better tolerated by the cardiovascular and neuromuscular system than emetine

[illegible]

associated with clinical symptoms

Headache was observed in 5 cases nausea in 4 vomiting in 3, abdominal pain in 8 diarrhoea in 7 and muscular pain in 4 cases

Local tolerance of Ro 1 9334 seems to be better than that of emetine In some cases the patients complained of the volume of liquid injected A 20% solution is therefore being prepared

To investigate the possibility of giving Ro 1 9334 *per os*, acid resistant-coated tablets were administered to a limited number of patients In spite of evident activity against *Schistosomiasis mansoni*, amoebiasis and isolated cases of infestation

with *Trichuris*, *Ascaris* and *Hymenolepis* the trials had to be stopped because of frequent nausea vomiting and diarrhoea A new form of tablet is under study

Summary

Chemical pharmacologic toxicologic and chemotherapeutic properties of Ro 1 9334 (2 dehydro emetine), a new synthetic compound are briefly discussed

As preliminary clinical trials in amoebiasis and schistosomiasis showed promising results, further clinical investigation of this drug is suggested

References

1 Osbond I M (1959) *Chem Ind* p 257
2 [illegible]
3 [illegible] ler
4 [illegible]
5 Bro s A (1959 [illegible] 104 592
6 [illegible]
7 [illegible]

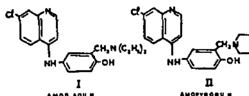
Annals of Biochemistry
and Experimental Medicine
Vol XX, 1960, Supplement

ANTI-AMOEBIC AGENTS PART V[1] PROMISING BASIC AMOEBICIDES DERIVED FROM 5 CHLORO 8-QUINOLINOL

J H BURCKHALTER, WILLIAM S BRINIGAR[2] AND PAUL E THOMPSON

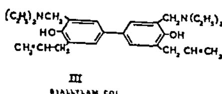
From the School of Pharmacy, University of Kansas, Lawrence, and the Research Division of Parke, Davis & Co, Detroit, Michigan

During the past fifteen years, we have been interested in the synthesis of phenolic Mannich bases as antiprotozoan agents Amodiaquin (camoquin) (I) (7 ...

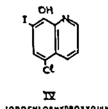


I AMODIAQUIN   II AMOPYROQUIN

administration (8) Biallylamicol (camoform) (III) was originally synthesized as an antimalarial (9) and it was found to be an effective agent against blood-induced human malaria (10) However, because of its activity against both intestinal and extraintestinal amœbiasis (11) it is being produced as an antiamœbic agent



III BIALLYLAMICOL



IV IODOCHLORHYDROXYQUIN

Iodochlorhydroxyquin (vioform) (IV) is illustrative of the 8 hydroxyquinoline amoebicides whose activity is limited to the intestine (12) In an attempt to convert the insoluble type IV to an agent which might possess systemic

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[1] Previous publication Nobles W L, Stephens, V C, Wei, L and Burckhalter, J H (1958), *J Amer Pharm Assoc*, 47: 82

[2] Parke, Davis & Co Fellow Chemical part was taken from his Ph.D Thesis, (1957) University of Kansas, U S.A

R₂N (CH₂)ₙNH-CH₂ — OH, N, Cl

VI

TABLE I

5-Chloro 7 (dialkylaminoalkylaminomethyl) 8-quinolinols

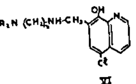
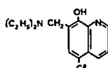
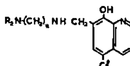
VI	R_2N	n	Procedure	Yield (%)	M p (°C)	Formula	Analyses C Calcd	C Found	H Calcd	H Found	Antiamoebic activity: *In Vitro*[r] (Min mal γ/cc)	Rats[s] (Approx CD_{50} mg/kg/day)	Dogs[t] (Approx CD_{50} mg/kg/day)
a	H_2N	0		80[a]	177	$C_{10}H_{10}ClN_3O$	53 70	53 93	4 51	4 67	< 20	425[u]	
b	$(CH_3)_2N$	0		40[b]	206 (d)	$C_{12}H_{14}ClN_3O$ 2HCl	44 38	44 08	4 97	5 18	< 20	>175	
c	$(C_2H_5)_2N$[c]	2									8		
d	$(C_2H_5)_2N$[d]	2	A	50[e]	188 (d)	$C_{17}H_{24}ClN_3O$ 3HCl 1/2H_2O	46 38	46 62	6 41	6 43	2 5	>675[u]	
e	$(CH_3)_2N$	3	A	20[f]	232 (d)	$C_{15}H_{20}ClN_3O$ 3HCl H_2O	42 77	42 71	5 98	5 96	5	150[u]	
f	$(C_2H_5)_2N$	3	A B	22[g] 39[i]	203 (d) 201 (d)	$C_{17}H_{24}ClN_3O$ 3HCl[h] $C_{17}H_{24}ClN_3O$ 2HCl	47 35 51 72	47 55 51 84	6 31 6 64	6 41 6 64	2 5	150	10
g	 [j]	3	A B	38[g] 35[k]	217 (d) 221 (d)	$C_{17}H_{22}ClN_3O$ 3HCl $C_{17}H_{22}ClN_3O$ 2HCl	47 57 51 98	47 70 52 00	5 87 6 16	5 87 6 17	<200	150	10
h		3	A	32[g]	223 (d)	$C_{18}H_{24}ClN_3O$ 3HCl	48 77	48 27	6 14	6 26	2 5	75	10
i	CH_3N	3	A	52[l]	264 (d)	$C_{18}H_{25}ClN_4O$ 3HCl	47 17	46 78	6 16	6 37	<200	225[u]	
j	$(C_2H_5)_2N$	4	A	45[b]	233 (d)	$C_{18}H_{26}ClN_3O$ 3HCl	48 55	48 42	6 56	6 67	<200	275[u]	
k		4	A	45[b]	208 (d)	C ,$H_{24}ClN_3O$ 3HCl H_2O	46 87	46 77	6 31	6 39	<200	275[u]	
l	$(C_2H_5)_2N$	4[m]	A	36[e]	236 (d)	$C_{19}H_{28}ClN_3O$ 3HCl[n]	49 68	49 77	6 80	7 23	10	275	>10

TABLE I (*Contd*)

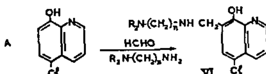
m	$(C_2H_5)_2N$[e]	5	A	30[a]	203 (d)	$C_{19}H_{28}ClN_3O$ 3HCl	49 68	49 74	6 80	6 84	<200	250	
n	(piperidino ring) N[e]	5	A	27[g]	237 (d)	$C_{20}H_{30}ClN_3O$ 3HCl H_2O	49 08	49 01	6 80	6 57	5		
o	$(C_2H_5)_2N$[q]	6	A	30[a]	205 (d)	$C_{20}H_{30}ClN_3O$ 3HCl	50 75	50 71	7 03	7 11	<200	175	~6 3

[a]Yellow solid from ethylene glycol monomethyl ether Nitrogen Calcd 18 79 Found 18 27 [c]Bright yellow crystals from methanol [d]See ref 16 for preparation [e]Contains an N methyl Side chain used N, N-diethyl N methylethylenediamine from Dr E F Elslager, Parke, Davis and Co [f]Yellow solid from absolute ethanol [g]Tan solid from ethanol methanol [h]Yellow solid from ethanol methanol [i]Ionic chlorine Calcd 21 67. Found 21 81 [j]White solid from ethanol [k]Intermediate diamine Cosse, J, Bryant J T and Shonle H A (1946), J Amer Chem Soc, 68: 1911 [l]White solid from ethanol methanol [m]Side chain actually from 4-diethylamino-1-methylbutylamine [n]White solid from dimethylformamide containing a small amount of water [o]Ionic chlorine Calcd 23 16 Found 23 36 [p]N, N Diethylcadaverine Kurtz, F (1951) *Ann*, 572: 23 made by the procedure of Ing, H R and Manske, R H F (1926) *J Chem Soc*, p 2348, [q]Kindly supplied by Dr E F Elslager, Parke, Davis and Co [r]N, N Diethyl-1,6-hexanethylenediamine, K N Campbell *et al*, *J Amer Chem Soc*, prepared by the method of Ing and Manske [s]Tested in a protein free liquid medium, usually for 48 hr, [t]Fed in the diet for 7 days [u]Given in gelatin capsules as 2 daily portions 5 days/week for 2 weeks, Thompson, P E *et al*, (1946) *Antibiotics & Chemotherapy* 6: 337 [v]Amounts requisite for appreciable effect closely approached the toxic levels





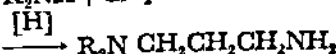
disproved the widely accepted theory of antiameobic action based upon the release of iodine which all the established 8-hydroxyquinolines contain However, a lack of satisfactory activity by type (V) compounds against hepatic amoebiasis in hamsters (14) suggested t in the side chain might be of such a compound (VI, wh (15), but the low order of ac further synthesis Nevertheless, continued unds of considerable promise One of them



methods in the literature or from com vas prepared by the addition of linpropionitrile The nitrile was nickel to obtain the diamine (B)



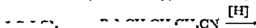
$$B \quad R_2NH+CH_2=CH\ CN \longrightarrow R_2N\ CH_2CH_2CN$$



$$\xrightarrow{[H]} R_2N\ CH_2CH_2CH_2NH_2$$

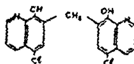
3-(4-methylpiperazinyl) propylamine was similarly prepared from 1 methylpiperazine

4 Diethylaminobutylamine and 4 pyrrolidinobutylamine were prepared by treatment of 3 chlorobutyronitrile with diethylamine and pyrrolidine, respectively,



$$\ldots CH_2CN \xrightarrow{[H]}$$

In general, reaction A proceeded smoothly. A small amount of byproduct 7:7'-methylene-*bis*-(5-chloro-8 quinolinol) (VII) usually formed in insignificant amount Previous studies have shown that VII may result from a reversal

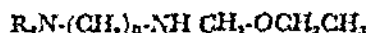


XII

of the Mannich reaction (15) The principal difficulty encountered in the

later stages of the preparations, it was found that the products could more easily be isolated by the addition of 2-molar equivalents of anhydrous hydrogen

before formation of the dihydrochloride increased the yields to 45 to 50% That the desired compounds were obtained in no greater yields is not surprising in view of the fact that primary amines in the Mannich reaction, in contrast to





VIII IX

through the formation of by-products

Attempts to synthesize (VI a and b) (Table I) by means of hydrazine and *unsym*-dimethylhydrazine in reaction A resulted in failure, with 5-chloro-8-quinolinol having been recovered unchanged Acetophenone likewise failed to undergo the Mannich reaction with *unsym*-dimethylhydrazine However, a successful Mannich-type condensation with hydrazine has been reported Frankel and Klager, obtained *sym*-*bis*-(2, 2-dinitropropyl)-hydrazine by the reaction of 2 2 dinitropropanol with hydrazine in glacial acetic acid (18) Hydrazine (VIa) was synthesized quite by accident In an effort to prepare the dihydrazide

... the compounds of type (X) were synthesized according to reaction D, and the results are summarized in Table II

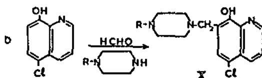
TABLE II

5-Chloro 7 (4 substituted 1 pipera inylmethyl) 8 quinolinols

X: R–N(piperazine)N–CH_2–(8-OH, 5-Cl quinolin-7-yl)

X	R	Yield, (%)	M p (°C)	Formula	Analyses C calcd	Analyses C Found	Analyses H Calcd	Analyses H Found	Anti amoebic activity In vitro[d] (Minimal γ/cc)	Anti amoebic activity Rats[e] (Approx CD_{50} mg /kg / day)
a.	CH_3	50	247 (d) 149	$C_{15}H_{18}ClN_3O$ 2HCl 1/2H_2O $C_{15}H_{18}ClN_3O$	48 21 61 74	48 49 61 12	5 66 6 22	5 75 6 23	5	>1000
b	$CH_3(CH_2$,	53	275 (d)	$C_{24}H_{36}ClN_3O$ 2HCl	58 72	58 55	7 80	7 70	< 200	350
c.	$C_2H_5O_2C$	77	133 188 (d)	$C_{17}H_{20}ClN_3O_3$ $C_{17}H_{20}ClN_3O_3$ 2HCl	58 37 48 30	58 34 48 04	5 76 5 25	5 30 5 70	2 5	>1250
d	H	82	343 (d) 230 (d)	$C_{14}H_{16}ClN_3O$ 2HCl 2H_2O[a] $C_{14}H_{16}ClN_3O$ 2H_2O	43 48 53 59	43 47 53 57	5 74 6 42	5 63 5 60	< 200	> 600
e	$(C_2H_5)_2NCO$	56	200 (d)	$C_{19}H_{25}ClN_4O_2$ 2HCl[b]	49 25	49 16	6 20	6 34	< 200	925
f	HC_2OCH_3	71	206	$C_{16}H_{18}ClN_3O_3$	57 23	57 27	5 40	5 63	< 200	350
g	$C_2H_5SO_2$	80	215 (d) 158	$C_{16}H_{20}ClN_3O$ S HCl $C_{16}H_{20}ClN_3O$ S	47 29 51 95	47 24 52 02	5 21 5 45	5 21 5 50	< 200	725
h	5-Cl-8-OH-7-CH_3 quinoline[c]								<2000	

[a] Anal for ionic chlorine Calcd 18 34 Found 18 30 [b] C H and H_2O analysis corresponds to 3/4 H_2O Calcd 3 75 Found 3 54 [c] See ref 16 for original synthesis [d] See footnote r of Table I [e] See foot note s of Table I



5-Chloro-7 1 piperazinylmethyl 8 quinolinol (Xd) was obtained by

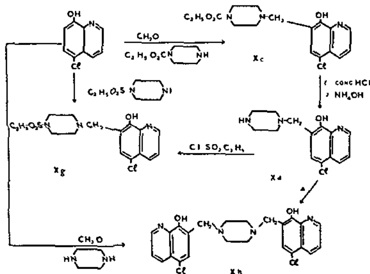


Fig 1

The compounds of Tables I and II

even as the dihydrochloride salts

18

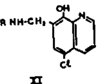


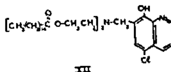
TABLE III

5-Chloro-7 (alkylam no nethyl) 8 quinolinoles

XI	R	Procedure	Yield (%)	M p °C	Formula	Analyses C Calcd	Analyses C Found	Analyses H Calcd	Analyses H Found	Antiamoebic activity In Vitro[d] (Mineral γ/cc)	Antiamoebic activity Rat[e] (Approx CD_{50} mg /kg day)
a	$CH_3(CH_2)_3CH(C_2H_5)CH_2$	A	43[a]	209 (d)	$C_{18}H_{25}ClN_2O \cdot 2HCl$	54 90	55 28	6 91	7 14	6 3	375
b	$CH_3(CH_2)_6$	A	53[b]	197 (d)	$C_{17}H_{23}ClN_2O \cdot 2HCl$	53 76	53 92	6 64	6 64	<200	625
c	$CH_3(CH_2)_7$[c]	A	41[b]	190 (d)	$C_{18}H_{25}ClN_2O \cdot 2HCl$	54 90	55 21	6 91	7 08	<200	>300

[a] Yellow solid from ethanol methanol [b] Yellow solid from absolute ethanol [c] Intermediate octylamine made from octyl bromide and potassium phthalimide by use of the general procedure of Sheehan J C and Bolhofer, W A (1950) *J Amer Chem Soc*, 72, 2786 [d] See foot note r, Table I [e] See foot note s Table II

A few other compounds which may not be conveniently listed in Tables I, II or III were synthesized 5 Chloro 7-[*bis* (2-hydroxyethyl)-aminomethyl]-8 quinolinol dipalmitate hydrochloride (XII) was obtained by the action of palmitoyl chloride upon 5 chloro-7-[*bis*-(2 hydroxyethyl)-aminomethyl] 8-



quinolinol Like the agents of Table III, it was virtually insoluble in water

When 5 chloro-8-quinolinol, paraformaldehyde and $\beta\beta$-iminodipropionitrile were allowed to heat at reflux temperature in alcohol, the expected 5-chloro-7-[*bis*-(2 cyanoethyl-aminomethyl]-8-quinolinol (XIII) was obtained (Fig 2) Treatment of (XIII) with boiling alkali failed to yield the corresponding diacid but instead gave 7, 7-methylene-*bis* (5-chloro-8 quinolinol) (VII), thus representing another example of the reversal of the Mannich reaction under [illegible]

isolated in both cases The product thus obtained in three different ways was assigned structure (XIV) It was somewhat surprising to find that complete esterification had occurred during the process of recrystallization of the diacid hydrochloride from alcohol

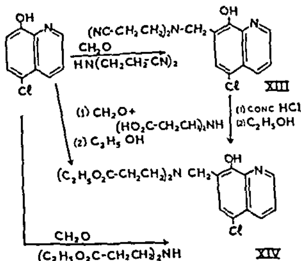
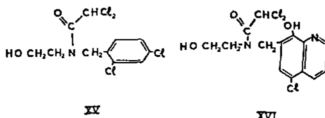
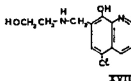


Fig 2

Upon learning the interesting anti amoebic activity of N (2,4 dichlorobenzyl)-N-(2-hydroxyethyl) dichloroacetamide (XV) in animals (21), we decided to attempt the synthesis of N (5 chloro 8-hydroxy 7 quinolylmethyl) N (2 hydroxyethyl) dichloroacetamide (XVI) Intermediate 2 (5-chloro 8 hydroxyquinolylaminomethylamino) ethanol (XVII) was prepared by use of ethanolamine in reaction D (XVII) was then allowed to react with methyl dichloroacetate to



give (XVI) Difficulty was encountered in this reaction owing to the low solubility of (XVII) in common organic solvents Reaction was finally effected by allowing a suspension of (XVII) in excess methyl dichloroacetate to stand for several weeks



Pharmacological results The anti-amoebic evaluation of these compounds was oriented around the primary goal of developing useful drugs rather than a [illegible]

substances were to be tested against hepatic amoebiasis in hamsters As past experience had suggested that greater emphasis be placed on *in vivo* performance than on *in vitro* potency, the endpoint of activity *in vitro* was determined for less than half of the compounds It also is to be emphasized that the effects of most of the compounds *in vivo* are based on relatively small numbers of animals and at best represent only rough approximations

Data on the anti amoebic activities are given together with a summary of the [illegible] Tables I, II and III Anti amoebic data on *Table IV* [illegible] I, were deemed worthy of trial in dogs [illegible] 1)—had in rats a therapeutic index (ratio of minimum effective to maximum tolerated dose) of two or more, and three of them cured infections in dogs without any gross evidence of toxicity It is to be noted that compounds with three methylene groups appeared to be superior to this with either two or four to six methylene groups

TABLE IX

Miscellaneous basic 5-chloro 8 quinolinols

Compound	Antiamoebic activity	
	in vitro[a] (Minimal γ/cc)	Rats[b] (Approx CD_{50} mg/kg/day)
XII	> 2000	> 2150
XIV	< 200	375
XVI	20	> 175
XVII	2.5	600

[a] See foot note r of Table I
[b] See foot note s of Table I

Compound (VII) of Table I [5-chloro 7 (3-diethylaminopropylaminomethyl)-8-quinolinol] appeared from several standpoints to be one of the most effective substances against experimental intestinal amoebiasis in rats and dogs It also proved to be as active as chloroquine (6) when tested orally against hepatic amoebiasis in hamsters These findings resulted in the decision to conduct a pre-clinical toxicity study of this substance, and the latter work has sustained interest in the compound as a possible anti-amoebic drug

SUMMARY

A continuation of the search for antiprotozoan agents among phenolic Mannich bases has led to the synthesis of a group of such bases from 5-chloro-8-quinolinol Several have been found to be highly effective against *Entamoeba histolytica in vitro* and against experimental intestinal amoebiasis in rats and dogs One of them, 5-chloro-7-(3-diethylaminopropylaminomethyl)-8 quinolinol (K SN 322), has been studied toxicologically and is now being investigated for anti-amoebic activity in man

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14 [illegible]

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Annals of Biochemistry
and Experimental Medicine
Vol XX, 1960, Supplement

ANTIAMOEBIC ACTION OF SUBSTITUTED QUINOLINES, QUINALDINES, QUINAZOLINES, QUINAZOLONES CHROMANONES, THIOCHROMANONES, DIAMINOALKANES, BENZYLAMINES AND CRESOLS

B S KAUSHIVA

From the Central Drug Research Institute Lucknow

This paper describes the testing of various compounds (1-8) for activity [illegible] with *Entamoeba* [illegible] ries, belonging [illegible] clinical value [illegible] have also been included for their usefulness in evaluating the methods and criteria employed and in elucidating the structure-activity relationships Emetine conessine, chiniofon and vioform served as reference drugs and their activity was almost in agreement with the findings of other workers

Materials and Methods

In vitro *tests* The EA strain of *E histolytica* obtained from Dr R A Neal, Wellcome Laboratories of Tropical Medicine, London, served as the test organism The general test procedure was similar to that described in earlier papers [illegible]

brought into solution or into emulsion with the help of organic solvents and [illegible]

[illegible]

of infection was 2 62 (range 1-4) and 2 66 (range 1 2-4) for abundance of amoebae and caecal lesions respectively

The experimental animals were albino rats of both sexes (25±5 g) maintained on stock diet of the C D R I Each rat was inoculated intracaecally with 0 5 cc of the inoculum (i e, 50,000—100,000 amoebae) The chemotherapeutic agents were administered 24 hr after the operation and the effect was investigated by oral administration of the drug for six consecutive days The

after seven days and the extent of infection was assessed on the basis of caecal lesions, the appearance of faecal contents, and presence and abundance of amoebae in caecal mucosa The state of the caecum of each rat was assessed by an arbitrary scoring on the basis of the following scheme

Caecal lesions (0=apparently normal caecum, 1=questionable or slight thickening, 2=moderate or restricted thickening, 3=marked thickening possibly with slight ulceration, 4=great thickening with much ulceration)

Abundance of amoebae [0=none, 1=very few (<1 in 20 fields), 2=few (1-5 per 20 fields), 3=moderately numerous (1-5 per field), 4=numerous (>10 per field))

Appearance of faecal contents (0=apparently normal 4=mucus and pus present in small quantities)

order

3=m

order of activity

Sources of test compounds Most of the compounds examined have been prepared at the Central Drug Research Institute (1 3) (RG, I, & BP series) and others have been received from Calcutta (4) BPC series), Lucknow (5, 6) (YDK and SLA series) and Vikram University (7, 8) (CNK series) The known compounds were purchased from commercial sources

Results and Discussion

In the present study, *in vitro* amoebicidal activity was found to be widely distributed among the various chemical series Thus 12 of 115 compounds were amoebicidal at concentration of 500 1000μg /cc, 35 at 100 500μ g /cc, 43 at 10 100 μg /cc and 25 at <10 μg /cc Out of 68 compounds that were active *in vitro* at 10 100 μg /cc or less, 35 were tested against intestinal infection in rats and 11 of these proved effective in rats in varying degrees In general, the correlation between *in vitro* and *in vivo* testing as revealed by a study of 53 compounds which were tested by both methods was poor, particularly in miscellaneous amines (Table VI) and chromanones (Table X) In most of the cases the compounds which were quite active *in vitro* were later found to be inactive *in vivo* However compounds I_{10} and I_{19} showed comparatively feeble *in vitro* activity but were slightly to moderately effective *in vivo* The *in vitro* tests provide a direct assessment of amoebicidal action of the compounds and yield useful information on the relation between structure and activity whereas the *in vivo* tests are valuable for assessing therapeutic possibilities

Quinaldines In Table I, the *in vitro* activity of all the compounds is fairly high excepting RG6 and 7 Perhaps the introduction of large branched groups

RG2 is not toxic and not active whereas RG11 is very toxic but not so very active

p Chlorophenylamino substitution at 4 position in the quinaldines yields compounds of promising activity (RG 1 and RG 12) The hydroxy derivative (RG 12) however, shows outstanding activity and has been selected for further studies

Quinolines The *in vitro* activity is not effected by varying the length of alkyl chain (YDK 1-4) In this series (Table II) perhaps the presence of polar sulphonic group has a damping effect on the *in vitro* activity of quinolines (YDK I 6) because this group usually results in unfavourable oil water partition coefficient, but slight *in vitro* activity is exhibited by YDK 5 and YDK 6 in which methyl-piperidino and morpholino substitutions have been made at 7 position However, both the compounds at 100 mg kg dose were inactive in rats In our experiments chiniofon (yatren manufactured by Bayer in Germany) shows slightly higher activity than crystallized vioform at 300 mg kg which may probably be due to experimental variations RG 15 which resembles vioform except that there is a $-OCH_3$ group at 8 position instead of $-OH$ group is quite active *in vitro* and *in vivo*

TABLE I

6 Methoxy 4 substituted amino 8 hydroxy (and methoxy) quinaldines

Screening test results against E histolytica in vitro and in rats

CH_3O — NH X — CH_3 HCl — R

COMPOUND NO	SUBSTITUENTS X	R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO MINIMUM AMOEBICIDAL CONC µg/ml
RG 4	n C_5H_{11}	OCH_3	00	100	0	6
RG 5	n C_7H_{15}	OCH_3	>160	00		8
RG 6	CH_3 $CH(CH_2)_3N(C_2H_5)_2$	OCH_3	>260	100	1	125
RG 7	$(CH_2)_3N(C_2H_5)_2$	OCH_3	>175	100 50	1 1	125
RG 2	⌬-OCH_3	OCH_3	>160	100	1,1	8
RG 1	⌬-Cl	OCH_3	>100	100 50	3,4 1	4
RG 3	n C_7H_{15}	OH	50 100	100/3 days [illegible]/3 days	2 3	10 100
RG 8	n-C_8H_{17}	OH	>175	80/3 days 25/3 days	0	4
RG 9	n $C_{10}H_{21}$	OH	>200	100	0	8
RG 10	CH_3-⌬	OH	>200	100	0	16
RG 11	⌬-OCH_3	OH	25	10	2	2 4
RG 12	⌬-Cl	OH	>200	100/3 days 50/3 days 50 0	4 <1>	2 4

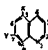
Quinazolones All the derivatives (Table III) show feeble *in vitro* activity except 182 in which benzyl has been substituted at 3 position and which is sufficiently active The compound I_{88} is highly active *in vivo* for the size of dose, compound I_{74} is also very active in rats but is rather toxic

Quinazolines In this series (Table IV) the *in vitro* activity is generally less than in the variously substituted quinazolones However, compounds I_{32} and I_{33} show fair activity *in vitro* and compounds I_{10} I_{19} and I_{33} show mediocre *in vivo* activity

Cresols Among the analogues of camoform (Table V) SLA 16, 19 and 22 are more active *in vitro* than SLA 18 21 and 23 Apparently the activity is sharply decreased as a result of tertiary butyl substitution It would, however, be interesting to compare the activity of tertiary butyl substituted compounds with normal butyl compounds The piperidino and morpholino substitution makes little difference in the activity

TABLE II

5 7 *Di substituted* 8 *hydroxy* (*and methoxy*) *quinolines*
Screening test results against E histolytica in vitro *and in rats*



COMPOUND NO.	SUBSTITUENTS X	Y	R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO MINIMUM AMOEBICIDAL CONC µg/ml
YDK 1	SO_3H	$-CH_2N(CH_3)_2$	OH	>200	-	-	1000
YDK 2	SO_3H	$-CH_2N.(C_2H_5)_2$	OH	>200	-	-	500-1000
YDK 3	SO_3H	$-CH_2-N(C_3H_7)_2$	OH	>140	-	-	1000
YDK 4	SO_3H	$CH_2-N.(C_2H_4OH)_2$	OH	>125	-	-	1000
YDK 5	SO_3H	CH_2 N	OH	>200	150 100	2 0	250
YDK 6	SO_3H	$-CH_2$ N	OH	>200	100	1	250
YATREN	SO_3Na	I	OH	-	300	5	125
VIOFORM	Cl	I	OH	-	300	3	10
RG15	Cl	I	OCH_3	>300	300	5	10

Miscellaneous amines The various amines and diamines synthesized on the basis of partial structure of emetine (BPC and CNK series, (Table VI) give high *in vitro* activity but are ineffective in rats In the 1 substituted benzylamino-2 piperidino (and morpholino) ethanes (Table VII) the piperidino and morpholino substitution does not make any appreciable difference in their *in vitro* activity The *in vitro* activity is still less in the various 1 substituted benzylamino-3 piperidino (and morpholino) propanes (Table VIII) but morpholino derivatives exhibit higher activity than piperidino substituents in this series None of the compounds

belonging to 1-piperidino 5 substituted aminopentanes (Table IX) evinced sufficient *in vitro* activity but compounds BP 6 BP 8 and BP 9 are slightly more active than the remaining ones This observation may tend to indicate that in this class of compounds the presence of 3 4-dialkyloxyphenyl substituents in the molecule may be important for activity However, *in vivo* activity in BP 8 is totally absent

TABLE III

3-Substituted 8 hydroxy (and methoxy) quinazolones
Screening test results against E histolytica in vitro *and in rats*

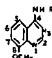


COMPOUND NO	SUBSTITUENTS X		R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO MINIMUM AMOEBICIDAL CONC µg/ml
I1	H		OCH3	> 150	–	–	100 – 125
I5	-CH3		-OCH3	> 260	100	2	>100
I69	C2H5		-OCH3	> 240	–	–	>100
I73	n-C3H7		-OCH3	> 33	33	1	>100
I78	n-C4H9		-OCH3	> 100	100	1	>100
I20	-CH2 CH2Cl		-OCH3	> 130	50	1	>100
I8	-H		-OH	> 220			>100
I6	CH3		-OH	> 100	100	2	>100
I10	-C2H5		-OH	> 220			>100
I16	n-C3H7		-OH	> 45	33	514	>100
I30	n-C4H9		OH	> 200	100	1	>100
I82	-CH2-(ring)		-OH	> 100	33 10	5 2(1)	14

Chromanones and thiochromanones The thiochromanones are found to be more active *in vitro* than the corresponding chromanones (Table X) and it would appear that thiochromanone nucleus itself may be a more desirable pharmacophore for the synthesis of amoebicidal compounds But the high *in vitro* activity in both the series bears little relationship to the *in vivo* activity which is low YDK 10, 11, 14 and SLA 11 and 12 (chromanones) are inactive in rats and similarly among thiochromanones YDK 20 and SLA 2 are inactive but YDK 21 has slight activity The activity in compounds of chromanone series varies from group to group as follows 6-I and 6 8 Cl are more active than 6 Cl 8 Cl are more active than 6-Cl, but 8 Cl and 8 OCH3 show almost similar activity and the piperidino substitution is slightly less active than the morpholino substituents In the thiochromanone series 6-OCH3 and 6-Cl or 8 OCH3 and 8-Cl do not show similar activity and no conclusive remarks can be made due to insufficient number of corresponding pairs in the series Similarly no decisive comments can be made

TABLE IV

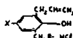
4 Substituted amino 8 methoxy quinazolines Screening test results against E histolytica in vitro *and in rats*



COMPOUND NO	SUBSTITUENTS R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO MINIMUM AMOEBICIDAL CONC µg/ml
1_{10}	n-C_4H_9	> 100	100	2	1000
1_{32}	n-C_5H_{11}	> 50	10	0	63
1_{38}	iso-C_5H_{11}	> 70	33	2	31-63
1_{19}	n C_6H_{13}	> 200	100	3	1000
1_{26}	$-C_6H_4-Cl$	> 60	50	0	500
1_{27}	$-C_6H_4-OCH_3$	> 100	100	1	1000
1_{31}	$CH_2-C_6H_5$	200	100	0	1000
1_{29}	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	> 100	100	0	250-500

TABLE V

2 Allyl 4 substituted 6 morpholino (and piperidino) methyl phenols Screening test results against E histolytica in vitro *and in rats*



COMPOUND NO	SUBSTITUENTS X	SUBSTITUENTS R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	IN VITRO MINIMUM AMOEBICIDAL CONC µg/ml
SLA 16	CH_3	N O (morpholino)	>100	100	3	63
SLA 17	-Cl	N O (morpholino)	>100	00	2	63
SLA 18	$-C(CH_3)_3$	N O (morpholino)	>100			250
SLA 19	$-CH_3$	N (piperidino)	>100	00	1	63
SLA 20	-Cl	-N (piperidino)	>100	100	0	16-31
SLA 21	$-C(CH_3)_3$	N (piperidino)	>100		-	1000
SLA 22	CH_3	$-N$ (piperazine) $N-CH_2$ (2-allyl-4-methyl phenyl; $CH_2CH=CH_2$, CH_3)	>100	100	0	31
SLA 23	$C(CH_3)_3$	N (piperazine) $N-CH_2$ (HO-, $CH_2-CH=CH_2$, CH_3 phenyl)	>100	-	-	250
CAMOFORM		[structure: $CH_2-CH=CH_2$, OH, $N(C_2H_5)_2$]	>100	100	2	53-125

whether alkyl substitution renders the compounds less active in comparison to the corresponding halogen substituted compounds or not

In the light of above observations it may be useful to make some comments regarding the mechanism of antiamoebic action among the compounds of quinoline ring system Some of the compounds included in this study have been tested in the Antibiotic Section of the Institute against a variety of bacteria and in preliminary screening are found to be inactive against them at 1 5000 dilution But

TABLE VI

Miscellaneous amines

Screening test results against E histolytica in vitro *and in rats*

Series A: CH_3O, CH_3O, $CH{\cdot}R$, NH_2HCl

Series B: CH_3O, CH_3O, $CH{\cdot}R$, $NH(CH_2)_3{\cdot}NH_2HCl$

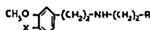
Series C: CH_3O, CH_3O, R, $CH{\cdot}NH_2HCl$, CH_3

Series D: CH_3O, CH_3O, R, $(CH_2)n{\cdot}NH_2HCl$

COMPOUND NO	SUBSTITUENTS n	SUBSTITUENTS R	MAXIMUM TOLERATED DOSE mg/kg	DOSE mg/kg	ORDER OF ACTIVITY	NV TRD MINIMUM AMOEBICIDAL CONC. mg/ML
SERIES A						
SPC 3		n-C_5H_{11}	> 100	100	1	8 16
SERIES B						
CNK 1		C_2H_5	> 75	–		12 5
CNK 2		n-C_3H_7	> 100			63
CNK 3		n-C_4H_9	> 100	100	1	31–63
CNK 4		n C_5H_{11}	> 100			16
SERIES C						
SPC 2	–	n C_6H_{13}	> 100	100	1	4
SERIES D						
SPC 1	2	n-C_6H_{13}	> 33	33	0	4
CNK 5	3	$-CH_3$	> 100			56
CNK 6	3	C_2H_5	> 100			16
CNK 7	3	n-C_3H_7	> 100	100	1(?)	16
CNK 8	4	n-C_3H_7	> 100	100	1(?)	8
CNK 8	4	n C_4H_9	> 100	100	0	4 8

TABLE VII

1-*Substituted benzylamino 2-piperidino (and morpholino) ethanes*
Screening test results against E histolytica in vitro



COMPOUND NO	SUBSTITUENTS		MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MINIMUM AMOEBICIDAL CONC μg/Ml
	X	R		
SLA 25	$-OCH_3$	- N	> 100	63—100
SLA 26	$-OCH_3$	- N	> 50	63-100
SLA 24	OC_4H_9	- N	> 100	100-125
SLA 28	$-OC_4H_9$	- N	> 100	100
SLA 29	OC_5H_{11}	- N	> 100	125
SLA 27	OC_5H_{11}	- N	> 70	500
SLA 30	OC_6H_{13}	- N	> 100	100-125

TABLE VIII

1-*Substituted benzylamino-3-piperidino (and morpholino) propanes*
Screening test results against E histolytica in vitro

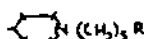


COMPOUND NO	SUBSTITUENTS		MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MINIMUM AMOEBICIDAL CONC μg/Ml
	X	R		
YDK 25	$COCHCl_2$ $N-CH_2$ Cl Cl	-N	>100	250
YDK 26	$COCHCl_2$ $-N-CH_2$ Cl Cl	N	>100	1000
YDK 27	$-HN-CH_2$ Cl Cl		>100	250
YDK 28	$-HN-CH_2$ Cl Cl	-N	>100	250-500
YDK 29	$-HN-CH_2$ OCH_3 OCH_3	-N	>100	125
YDK 30	$-HN-CH_2$ OCH_3 OCH_3	-N	>100	500

being the most active compounds In 4 substituted 8 methoxy quinazolines absence of activity may be due to lack of chelation but the activity in I_{32} and

TABLE IX

1 *Piperidino 5 substituted aminopentanes*
Screening test results against E histolytica in vitro



COMPOUND NO	SUBSTITUENTS R	MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MINIMUM AMOEBICIDAL CONC. µg/Ml
BP1(202)	-NH CH_3	> 125	100
BP2(204)	-NH-C_2H_5	> 150	100
BP4(205)	NH CH(CH_3)(CH_3)	> 150	100
BP5(206)	NH C_4H_9	> 140	100
BP6(210)	NH CH_2-C6H4-OCH_3	> 150	100
BP7(208)	NH-CH_2-C6H3-(OCH_3)$_2$	> 125	100
BP8(209)	-NH CH_2-C6H3-(OC_2H_5)$_2$	>150	100 (150 mg/kg 6 days, O)*
BP9(211)	NH H_2C-furyl	>125	100
BP3(212)	N(CH_3)(CH_3)	>130	100

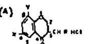
*Indicates that four rats were treated at 150 mg /kg for 6 days and showed an order of activity equal to 0

concerned in subscribing towards the activity of compounds.

TABLE X

Substituted (1) *chromanones and* (B) *thiochromanones*
Screening test results against E histolytica in vitro

(A)



COMPOUND NO	SUBSTITUENTS X Y	R	MAXIMUM TOLERATED DOSE mg/kg	IN VITRO MINIMUM AMOEBICIDAL CONC µg/ml
YDK 7	6 Cl	-N(CH₃)2	> 150	31
YDK 8	6 Cl	N-ring	> 130	3
YDK 9	6 – Cl	N-ring	> 120	63
YDK 16	6 – Cl	NH CH-ring	> 200	125
SLA 14	6 – I	N(CH₃)₂	> 2 0	8
SLA 11	6 – I	N-ring	> 100	8 (100 mg/kg/6 days)
SLA 13	6 – I	N-ring	–	8 16
SLA 15	6 – I	NH CH₂-ring	> 100	8
YDK 10	8 – Cl	N (CH₃)₂	> 175	8 (mg/kg/6 days)
YDK 11	8 – Cl	N-ring	> 120	8 – 16 (100 mg/kg/6 days [illegible])
YDK 12	8 Cl	N-ring	> 100	8
SLA 12	8 – I	N-ring	> 130	8 16 (100 mg/kg/6 days 0)
SLA 1	8 – OCH₃	N-ring	–	8 – 16
SLA 3	8 – OCH₃	N-ring	–	8
YDK 13	6,8 – Cl	N (CH₃)₂	> 200	8
YDK 14	6 8 – Cl	N-ring	> 140	4 – 8 (100 mg/kg/6 days 0)
YDK 15	6 8 – Cl	N-ring	> 170	8

(B)



YDK 17	6 – Cl	N(CH₃)₂	> 100	8
YDK 18	6 – Cl	-N-ring	> 160	16 — 31
YDK 19	6 – Cl	N-ring	> 160	16
SLA 1(a)	6 OCH₃	N-ring	—	31 (100 mg/kg/6 days 0) *
YDK 20	8 – Cl	N (CH₃)₂	> 150	16 (100 mg/kg/6 days 1) *
YDK 21	8 – Cl	-N-ring	> 130	4 (100 mg/kg/6 days 2) *
YDK 22	8 – Cl	-N-ring	> 130	4
YDK 23	8 – H	N-ring	> 130	8
YDK 24	8 – H	-N-ring	> 200	8 — 16
SLA 5	8 – CH₃	N-ring	> 100	31
SLA 7	8 – CH₃	N-(CH₃)₂	> 220	16
SLA 9	8 – CH₃	NH CH₂-ring	—	8 — 16
SLA 2	8 – OCH₃	N-ring	> 100	31 (100 mg/kg/6 days 0) *
SLA 4	8 – OCH₃	N-ring	—	31
SLA 6	8 – C₂H₅	N-ring	—	16
SLA 8	8 – C₂H₅	N (CH₃)₂	—	6 — 31
SLA 10	8 – C₂H₅	NH CH₂-ring	> 100	16 — 31

* SAME AS IN FOOTNOTE TO TABLE X

Summary

The amoebicidal activity of 115 substituted quinolines, quinaldines, quinazolines, quinazolones, chromanones thiochromanones, diaminoalkanes, benzylamines and cresols has been tested against *E histolytica in vitro* and 53 compounds were tested in intestinal amoebiasis in rats

Am

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activity both *in vitro* and *in vivo*

In the quinazolone series (Table III) compounds I_{78} and I_{82} and in quinazoline series (Table IV) compounds I_{10} I_{19}, and I_{38} are fairly active

6 Morpholino methyl 2 allyl-4 methylphenol (SLA 16) and 6 morpholino methyl 2 allyl-4 chlorophenol (SLA 17) which have been prepared as analogs of camoform show activity comparable to camoform In this series, tertiary butyl substitution, however, decreases the activity sharply

Some of the amines and diamines synthesized on the basis of partial structure of emetine show considerable *in vitro* activity but they are less active *in vivo* (Table

substitution

The thiochromanones are more active than the corresponding chromanones (Table X) but the high *in vitro* activity found in these series is not reflected in the experimental amoebiasis in rats

Acknowledgements

The author is highly grateful to Dr F Hawking, Colombo Plan Expert, for stimulating criticism and continued interest in the work Thanks are also due to Drs B Mukerji M L Dhar and B N Singh for advice and encouragement to Drs B Pathak, C N Kachru, Y D Kulkarni R N Iyer S L Arora and R Gopalachari for the supply of drug samples and to Mr A K Sarkar and Mr V K Misra for technical assistance

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J Trop Med Hyg **4** 224

Annals of Biochemistry
and Experimental Medicine
Vol XX, 1960, Supplement

POTENTIAL AMOEBICIDES PART VIII SYNTHESIS OF MANNICH BASES OF 4, 5, 6-, AND 7 HYDROXYQUINOLINES, 4 HYDROXYQUINALDINE, AND 1 2 3 4 TETRAHYDRO 6 HYDROXYQUINOLINE

J S TANDON R N IYER AND R GOPALACHARI

From the Central Drug Research Institute Lucknow

teability *per se* is obviously not necessary for such deactivation for otherwise one

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quinoline have now been synthesized and are reported in this present communication In all these compounds the relative positions and the spatial disposition of the side chain nitrogen functions and of the phenolic residues are such as to permit facile chelation

It may be pointed out that with the extra basic centre in the molecule these new compounds may tend to facilitate the formation of chelate complexes at a *p*H slightly higher than 7 *cf* Phillips *et al* (2) This would constitute an

contents (and of the biological

be above 7 Thompson

nomethyl-8-hydroxyquinoline

than 5 7-diiodo-8 hydroxy-

ity may be an expression of

the facile chelation of this compound in the lower regions of the intestine because of its higher basicity Since however, this compound is derived from 8-hydroxyquinoline its use as a systemic amoebicide is likely to be limited due to its deactivation by the intact red cell wall

Burckhalter *et al* (4) have reported the synthesis of 7-diethylamino (and piperidino) methyl 8 hydroxyquinolines and of 8-diethylaminomethyl 7 hydroxyquinoline by a procedure involving the heating of an equimolecular mixture of the hydroxy compound, formaldehyde and the amine on the water bath Following their procedure we were unable to obtain our compounds in a pure form The products obtained in this working appeared to be mixtures from which only the corresponding bisquinolylmethanes could be isolated in a pure form. If, however, the reaction is carried out at the room temperature or even slightly lower than that the required products are obtained in good yields

5 Hydroxyquinoline was prepared by the reversed Bucherer reaction from 5 aminoquinoline (5) Experimental details of this reaction are included in th s paper beca

tion, in a 50 % yield

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hydr... obtained in 95 % yield by the reduction of hydroxyquinoline with hydrogen in the presence of Raney nickel (W4) (8) at a pressure of 50 atmosphere

The Mannich bases of 5 6 and 7 hydroxyquinolines are generally low melting solids or viscous liquids They are unstable and decompose slowly into the corresponding bisquinolylmethanes They form sharp melting picrates which are soluble in chloroform benzene and petrol ether in that order The Mannich bases of 4 hydroxyquinoline and 4 hydroxyquinaldine however are more stable The structures of the Mannich bases obtained from 4 hydroxy quinaldine are considered by analogy *cf* Price and Jackson (9) to be the normal 3 substituted compounds instead of 2 β aminoethyl derivaties

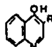
The compounds now reported are under investigation for their amoebicidal activity in the Division of Microbiology of this Institute The results will be communicated later

Experimental

5 Hydroxyquinoline 5 Aminoquinoline (obtained by the reduction of 10 g of 5 nitroquinoline (10) was refluxed with sodium metabisulphite (98% pure, 60 g) in water (80 cc) for 20 hr The reaction mixture was cooled and treated with aqueous sodium hydroxide (10%) to *p*H 10 and extracted with benzene to remove the unreacted amine The clear yellow aqueous solution was heated

TABLE I

Mannich bases from 4 hydroxyquinoline

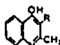


S NOS	R	MOLECULAR FORMULA	M P °C* HYDROCHLORIDE	FOUND N	REQUIRED H
250	$-CH_2-N(n\text{-}C_4H_9)_2$	$C_{18}N_{26}N_2O$	168 (d)	9 4	9 8
251	$-CH_2-N$⟨ring⟩	$C_5H_8N_2O$	205 (d)	11 3	11 6
252	$-CH_2-N$⟨ring⟩O	$C_{14}H_{16}N_2O_2$	160 (d)	11 4	11 5

* MELTING POINTS WERE DETERMINED IN SEALED CAPILLARIES AS THESE COMPOUNDS WERE FOUND TO BE VERY HYGROSCOPIC

TABLE II

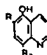
Mannich bases from 4 hydroxyquinaldine



S NOS	R	MOLECULAR FORMULA	M P °C	FOUND C	FOUND H	FOUND N	REQUIRED C	REQUIRED H	REQUIRED N
253	$-CH_2-N(C_2H_5)_2$	$C_{5}H_{20}N_2O$	324	74·0	7 8	11 5	73 8	8 2	11 5
254	$-CH_2-N(n\text{-}C_4H_9)_2$	$C_{19}H_{26}N_2O$	310	76 3	9 8	9 7	76 0	9 3	9 3
255	$-CH_2-N$ (piperidine ring)	$C_{6}H_{20}N_2O$	302	74 5	8 0	10 6	75 0	7 8	10 9
256	$-CH_2-N$ (ring) O	$C_{15}H_{8}N_2O_2$	320	70 0	7 2	10 8	69 8	7 0	10 8

TABLE III

Mannich bases from 5 hydroxyquinolines



S NOS	R	R_2	MOLECULAR FORMULA	M P °C	FOUND C	FOUND H	FOUND N	REQUIRED C	REQUIRED H	REQUIRED N
257	$CH_2N(C_2H_5)_2$	H	$C_{4}H_{18}N_2O$	VISCOUS OIL PICRATE 122	72 9	8 4	12 6	73 0	7 8	12 2
258	$-CH_2N$ (ring)	H	$C_{15}H_{18}N_2O$	OIL PICRATE 179	74 6	8 4	11 8	74 4	7 4	11 6
259	$-CH_2N$ (ring) O	CH_2N (ring) O	$C_{19}H_{23}N_3O_3$	169	66 3	7 2	12 0	66 5	7·3	12 2

5 Hydroxyquinoline was prepared by the reversed Bucherer reaction from 5 aminoquinoline (5) Experimental details of this reaction are included in this paper because the details of the procedure are not available in the literature 6- and 7-Hydroxyquinolines were obtained from *para* and *meta* anisidines, respectively, by Skraup synthesis as modified by Strukov (6), followed by demethylation, in a 50% yield

to

hyc

yield either by this procedure or by the use of sodium and alcohol or tin and hydrochloric acid It was finally obtained in 95% yield by the reduction of *hydroxyquinoline with hydrogen* in the presence of Raney nickel (W4) (8) at a pressure of 50 atmosphere

The Mannich bases of 5 6 and 7 hydroxyquinolines are generally low melting solids or viscous liquids They are unstable *and decompose slowly into* the corresponding bisquinolylmethanes They form sharp melting picrates which are soluble in chloroform benzene, and petrol ether in that order The Mannich bases of 4 hydroxyquinoline and 4 hydroxyquinaldine, however, are *more stable* *The structures* of the Mannich bases obtained from 4 hydroxy quinaldine are considered by analogy *cf* Price and Jackson (9), to be the normal 3 substituted compounds instead of 2 β aminoethyl derivaties

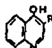
The *compounds now reported* are *under* investigation for *their* amoebicidal activity in the Division of Microbiology of this Institute The results will be communicated later

Experimental

5 *Hydroxyquinoline* 5 Aminoquinoline (obtained by the reduction of 10 g of 5 nitroquinoline (10) was refluxed with sodium metabisulphite (98% pure, 60 g) in water (80 cc) for 20 hr The reaction mixture was cooled and treated with aqueous sodium hydroxide (10%) to *p*H 10 and extracted with benzene *to remove* the unreacted amine *The clear yellow aqueous solution* was heated

TABLE I

Mannich bases from 4 hydroxyquinoline

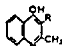


S NOS	R	MOLECULAR FORMULA	M P °C* HYDROCHLORIDE	FOUND N	REQUIRED H
250	$-CH_2-N(n\ C_4H_9)_2$	C_{18} N_{24} N_2 O	168 (d)	9 4	9 8
251	$-CH_2-N$	C_5 H_{18} N_2 O	205 (d)	11·3	11·6
252	$-CH_2-N$ O	C_{14} H_{16} N_2 O_2	160 (d)	11 4	11 5

* MELTING POINTS WERE DETERMINED IN SEALED CAPILLARIES AS THESE COMPOUNDS WERE FOUND TO BE VERY HYGROSCOPIC

TABLE II

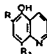
Mannich bases from 4 hydroxyquinaldine



S NOS	R	MOLECULAR FORMULA	M P °C	FOUND			REQUIRED		
				C	H	N	C	H	N
253	$-CH_2-N(C_2H_5)_2$	$C_{15}H_{20}N_2O$	324	74 0	7 8	11 5	73 8	8·2	11 5
254	$-CH_2-N(n\text{-}C_4H_9)_2$	$C_{19}H_{28}N_2O$	310	76 3	9 8	9 7	76 0	9 3	9 3
255	$-CH_2-N$(ring)	$C_{16}H_{20}N_2O$	302	74 5	8 0	10 6	75 0	7 8	10·9
256	$-CH_2-N$(ring)O	$C_{15}H_{18}N_2O_2$	320	70·0	7·2	10 8	69 8	7 0	10 8

TABLE III

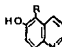
Mannich bases from 5-hydroxyquinolines



S NOS	R	R_2	MOLECULAR FORMULA	M P °C	FOUND			REQUIRED		
					C	H	N	C	H	N
257	$-CH_2-N(C_2H_5)_2$	H	$C_{14}H_{18}N_2O$	VISCOUS OIL PICRATE 122	72 9	8 4	12 6	73 0	7 8	12 2
258	$-CH_2-N$(ring)	H	$C_{15}H_{18}N_2O$	OIL PICRATE 179	74 6	8 4	11 8	74 4	7 4	11·6
259	$-CH_2-N$(ring)O	CH_2N(ring)O	$C_{19}H_{23}N_3O_3$	169	66 3	7 2	12 0	66 5	7·3	12·2

TABLE IV

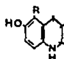
Mannich bases from 6 hydroxyquinoline



S NOS	R	MOLECULAR FORMULA	M P °C	FOUND C	FOUND H	FOUND N	REQUIRED C	REQUIRED H	REQUIRED N
260	$CH_2-N(C_2H_5)_2$	$C_4H_8N_2O$	VISCOUS OIL PICRATE 192	73 1	7 6	11 7	73 0	7 8	12 2
261	CH_2-N	$C_5H_6N_2O$	85°C PICRATE 204	74 0	7 5	11 1	74 4	7 4	11 6
262	CH_2-N O	$C_{14}H_6N_2O_2$	96–98	68 8	6 7	11 3	68 8	6 6	11 5
263	CH_2 N OH	$C_9H_4N_2O_2$ 2HCl	HYDROCHLORIDE 340(d)	60 4	4 0	7 6	60 5	3 5	7 5

TABLE V

Mannich bases from 6 hydoxy 1, 2 3 4 tetrahydroxyquinoline



S NOS	R	MOLECULAR FORMULA	M P °C	FOUND C	FOUND H	FOUND N	REQUIRED C	REQUIRED H	REQUIRED N
264	$-CH_2-N(C_2H_5)_2$	$C_{14}H_{22}N_2O$	VISCOUS LIQ	71 8	9 0	11 5	71 8	9 4	11 9
265	$-CH_2-N$	$C_{15}H_{22}N_2O$	SOLID 172–3	73 6	9 3	11 0	73 2	8 9	11 4
266	$-CH_2-N$ O	$C_{14}H_{22}N_2O_2$	VISCOUS LIQ	67 4	8 7	11 2	67 7	8 9	11 3

on a water-bath for 3-5 hr keeping the pH at 10 by occasional addition of alkali. This was then acidified with aqueous acetic acid (10%) to pH 6 to obtain the pale brown precipitate of 5-hydroxyquinoline, m p 222°C (lit 224°C). More of this compound could be recovered from the filtrate by basification followed by heating and acidification Total yield, 4 5 g

Mannich bases from hydroxyquinolines The preparation of these is illustrated by the procedure for the preparation of 6-diethylaminomethyl-5 hydroxyquinoline. Minor modifications, wherever necessary, have been indicated The final bases are listed in Tables I to VI

TABLE VI

Mannich bases from 7-hydroxyquinoline



S. NOS	R	MOLECULAR FORMULA	M P °C	FOUND C	FOUND H	FOUND N	REQUIRED C	REQUIRED H	REQUIRED N
267	$-CH_2N(C_2H_5)_2$	$C_{14}H_{18}N_2O$	VISCOUS LIQ PICRATE 190-192	72 7	8 2	12 5	73 0	7 8	12 2
268	$-CH_2N$ (piperidino)	$C_{15}H_{18}N_2O$	VISCOUS LIQ PICRATE 206-208	74 4	7 4	11 4	74 4	7 4	11 6
269	$-CH_2N$ (morpholino)	$C_{14}H_{16}N_2O_2$	VISCOUS LIQ PICRATE 216-217	68 3	6 8	11 9	68 8	6 6	11 5
270	$-CH_2-$ (hydroxyquinolyl, HO)	$C_{19}H_{14}N_2O_2$	237 238	75 3	4 6	9 3	75 5	4 6	9 3

6-Diethylaminomethyl-5-hydroxyquinoline (S No 257)

5-Hydroxyquinoline (1 5 g) was dissolved in ethanol (50 cc.) cooled at 4°C and diethylamine (1 2 cc, 1 mole) was added A solution of formaldehyde

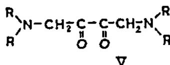
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obtained in a pure form by removing ethanol from the reaction mixture and crystallising from a suitable solvent. Chromatography is not necessary for their purification

Serial nos 250—252 were characterised as hydrochlorides (nos. 260, 264, and 267) were prepared by using 3 moles each of formaldehyde and the amine per mole of the hydroxy compound.

SUMMARY

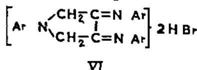
Synthesis of Mannich bases of 4-, 5-, 6- and 7-hydroxyquinolines, 4-hydroxyquinaldine and 1 2 3 4 tetrahydro-6-hydroxyquinoline as potential amoebicides is described



V

Compounds of this type were prepared by reacting the appropriate secondary amines with 1 4 dibromo 2 3 butane dione the latter being obtained by bromination of diacetyl (4)

Reaction of 1 4 dibromo 2 3 butanedione with aromatic primary amines proceeded in a d fferent manner whereby the di anils (VI) of 1 aryl 3 4 pyrrolidinedione were obtained Similar anils were also reported by Lehr *et al* (5 6 7) who obtained such compounds by condensing diacetyl with various primary amines in a search for anti tubercular drugs



VI

Reaction of 1 4 dibromo 2 3 butanedione with pyrrole hydrazine and ethylene diamine gave dark resinous products Reaction with piperazine N methyl and N ethyl aniline *o* phenylenediamine and 2 aminothiazole gave hygroscop c products which could not be analyzed satisfactorily Reaction

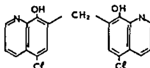
4 7 phenan

V) can also

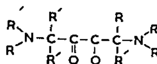
paration of

compounds of the type (VIII) where R —Me Et or Ph is in progress and this work will be published later

The biological screening of these compounds is in progress



VII



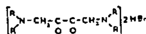
VIII

Experimental

All melting points are uncorrected

absolute alcohol and ether Most of the compounds are very hygroscopic.

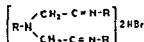
TABLE I



No	R	Mol formula	M p °C	Analysis N (%) Found	Analysis N (%) Required
1	Methyl	$C_8H_{18}N_2O_2Br_2$	Hygroscopic		
2	Ethyl	$C_{12}H_{26}N_2O_2Br_2$	Darkens at 150, melts at 172(d)	7.1	7.2
3	*n* Propyl	$C_{16}H_{34}N_2O_2Br_2$	259-60 (d)	7.0	6.7
4	*n* Butyl	$C_{20}H_{42}N_2O_2Br_2$	Darkens at 140, melts at 318 (d)	5.8	5.8
5	*iso*-Butyl	$C_{20}H_{42}N_2O_2Br_2$	308 (d)	5.9	5.8
6	*n* Amyl	$C_{24}H_{50}N_2O_2Br_2$	Darkens at 200 and *starts decomposing*	4.6	5.0
7	*iso* Amyl	$C_{24}H_{50}N_2O_2Br_2$	Darkens at 190 melts at 312 (d)	5.1	5.0
8	*Cyclo* Hexyl	$C_{28}H_{50}N_2O_2Br_2$	>320	4.9	4.6
9	Benzyl	$C_{32}H_{34}N_2O_2Br_2$	250	4.9	4.4
10	Piperidyl	$C_{14}H_{26}N_2O_2Br_2$	Starts decomposing at 139	7.2	6.8
11	Morpholyl	$C_{12}H_{22}O_4N_2Br_2$	Darkens at 170 melts at 199 (d)	6.6	6.7
12	1, 2, 3, 4 Tetrahydro quinolyl	$C_{22}H_{26}N_2O_2Br_2$	166 (d)	6.0	5.5
13	Ethyleneimino	$C_8H_{14}N_2O_2Br_2$	110 (d)	8.9	8.5
14	Indolyl	$C_{10}H_{18}N_2O_2Br_2$	315 (d)	6.1	5.8
15	2 Aminopyridyl	$C_{14}H_{16}N_4O_2Br_2$	*Effervesces with decomposition at 115-16°*	*13.6*	*13.0*

d = decomposition

TABLE II



No	R	Mol formula	M p °C	Analysis N (%) Found	Analysis N (%) Required
1	Phenyl	$C_{22}H_{21}N_3Br_2$	225-26	8.5	8.6
2	*p*-Chlorophenyl	$C_{22}H_{18}N_3Cl_3Br_2$	215 (d)	6.8	7.0
3	*p*-Methoxyphenyl	$C_{25}H_{27}O_3N_3Br_2$	211-12 (d)	6.6	7.2
4	*p*-Methylphenyl	$C_{25}H_{27}N_3Br_2$	285 (d)	7.1	7.9
5	β Phenylethyl	$C_{28}H_{33}N_3Br_2$	234-36 (d)	6.4	7.3
6	Benzyl	$C_{25}H_{27}N_3Br_2$	Softens at 167 melts at 188 (d)	7.3	7.8
7	*p*-Methoxybenzyl	$C_{28}H_{33}N_3Br_2$	189-90 (d)	6.2	6.7
8	*p-Diphenyl*	$C_{40}H_{33}N_3Br_2$	*316-17 (d)*	5.7	5.8
9	*cyclo*-Hexyl	$C_{22}H_{39}N_3Br_2$	Softens at 161 melts at 172-73 (d)	8.2	8.3
10	2 5-Dichlorophenyl	$C_{22}H_{15}Cl_6N_3Br_2$	243 (d)	5.9	6.0
11	*o*-Methoxyphenyl	$C_{25}H_{27}O_3N_3Br_2$	181-82 (d)	6.9	7.2
12	α Naphthyl	$C_{34}H_{27}N_3Br_2$	269 (d)	6.9	6.5
13	β-Naphthyl	$C_{34}H_{27}N_3Br_2$	209-10 (d)	6.1	6.5
14	*m* Chlorophenyl	$C_{22}H_{18}N_3Cl_3Br_2$	197-98 (d)	6.5	7.0
15	Benzidine	$C_{40}H_{36}N_6Br_2$	239-40 (d)	9.7	9.7
16	DDS	$C_{40}H_{36}O_6N_6Br_2S_3$	Effervesces at 216 (d)	7.4	7.2

Summary

Several 2 3 butanediones carrying tertiary nitrogen atoms in 1 and 4 positions have been synthesized as potential amoebicidal agents Reaction of 1 4 dibromo 2 3 butanedione with various aromatic primary amines gave the corresponding 3 4 dianils of 1 aryl 3 4 pyrrolidinedione

Acknowledgment

Thanks are due to Shri P N Khanna for microanalyses

References

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and Experimental Medicine

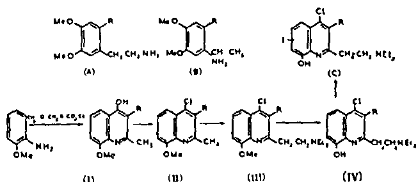
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SEARCH FOR NEW AMOEBICIDES PART IV

K L PATHAK AND B PATHAK

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Some halo-8-hydroxyquinolines, like chinifon, vioform and diodoquine possess pronounced amoebicidal activity Burckhalter and Edgerton (1) observed high *in vitro* amoebicidal activity in 5-chloro-7-diethylaminomethyl 8-quinolinol It was observed by Kaushiva (2) that high *in vitro* amoebicidal activity exists among compounds of the types A and B where R=*n* butyl or *n* hexyl In view of these observations it has been considered worthwhile to synthesise and investigate amoebicidal activity in compounds of the type C These compounds have been



[illegible]

pound C R n $C_{16}H_{13}$ could not be crystallized

EXPERIMENTAL

(Melting points are uncorrected Petroleum ether used had the b p 60-8)

2 3 *Dimethyl 4 hydroxy-8 methoxyquinoline* (I, R CH_3)

A mixture of *o*-anisidine (1 mol) 2 methyl-acetoacetic ester (1 mol) and few drops of dilute HCl was kept overnight and then heated in an oil-ba at

120 to 130° in a Dean and Stark apparatus till no more water collected Benzene was then distilled off and the residual viscous mass was poured into boiling diphenyl oxide After boiling for 15 min, the mass was cooled, diluted with petroleum ether, kept overnight, filtered and washed with fresh petroleum ether

The yield was 55% It was crystallized from alcohol in white needles, m p 289-91° (Found C, 70 5, H, 6 6 $C_{12}H_{13}O_2N$ requires, C, 70 94, H, 6 4%)

2-Methyl 3 ethyl-4 hydroxy-8-methoxyquinoline (I, $R=C_2H_5$)

It was prepared from a mixture of *o*-anisidine and 2-ethyl acetoacetic ester in 65% yield It was crystallized from alcohol in white needles, m p 288° (Found C, 71 5, H, 6 7 $C_{13}H_{15}O_2N$ requires C, 71 88, H, 6 91%)

2-Methyl-3 n butyl 4 hydroxy 8 methoxyquinoline (I, $R=C_4H_9$)

It was prepared from a mixture of *o* anisidine and 2-*n*-butyl-acetoacetic ester *in 52% yield It separated from alcohol in white needles, m p 196 97°* (Found C, 73 1, H, 7 5 $C_{15}H_{19}O_2N$ requires C, 73 47 H, 7 75%)

2-Methyl 3 n *amyl-4-hydroxy-8 methoxyquinoline* (I, $R=C_5H_{11}$)

It was prepared from *o*-anisidine and 2 *n* amyl-acetoacetic ester in 55% yield, white needles (alcohol), m p 165° (Found C, 73 9, H, 7 9 $C_{16}H_{21}O_2N$ requires C, 74 13, H, 8 12%)

2-Methyl 3-n-*hexyl-4-hydroxy-8-methoxyquinoline* (I, $R=C_6H_{13}$)

It was prepared from *o*-anisidine and 2 *n* hexyl acetoacetic ester in 42% yield It crystallized from alcohol in white needles, m p 152° (Found C, 74 4, H, 8 1 $C_{17}H_{23}O_2N$ requires C, 74 71, H, 8 42%).

2 3 *Dimethyl-4 chloro 8 methoxyquinoline* (II, $R=CH_3$)

A mixture of I ($R=CH_3$, 15g), $POCl_3$ (35 cc) and PCl_5 (3 g) was heated in an oil-bath at 125 30° for 3 hr The product was then cooled and poured on to ice The aqueous solution was partly neutralized, purified with charcoal and basified with ammonia under cooling The chloro compound separated out as fine needles and was crystallized from petroleum ether in colourless fine needles, m p 132-33°, yield, 14 g (Found C, 64 8, H, 5 3 $C_{12}H_{12}ONCl$ requires C, 65 01, H, 5 41%)

Its *picrate* crystallized from alcohol in deep yellow needles, m p 204 206°

2-Methyl 3 ethyl 4-chloro 8 methoxyquinoline (II, R C_2H_5)

Crystallized from petroleum ether in colourless fine needles, m p 115° (Found C, 66 0, H, 5 9 $C_{13}H_{14}ONCl$ requires C, 66 24 H, 5 94%) The *Picrate* crystallized from alcohol in yellow needles, m p 163-65°

2 *Methyl* 3-n-*propyl 4-chloro 8 methoxyquinoline* (II, $R-C_3H_7$)

Crystallized from petroleum ether in colourless needles, m p 122 23° (Found C, 67 0, H, 6 1 $C_{14}H_{16}ONCl$ requires C, 67 33, H, 6 41%) The *Picrate* crystallized from alcohol in yellow needles, m p 165-66°

7° (Found

The *Picrate*

2-Methyl-3 n-*amyl-4-chloro 8-methoxyquinoline* (II, $R-C_5H_{11}$)

Crystallized from petroleum ether in colourless needles, m p 101-102°.

(Found C 68 7 H 7 0 $C_{16}H_{20}O$ NCl requires C, 69 18 H 7 2%) The *picrate* crystallized from alcohol in yellow needles m p 161 62°

2 *Methyl* 3 n *hexyl* 4 *chloro* 8 *methoxyquinoline* (II $R-C_6H_{13}$)

Crystallized from petroleum ether in colourless needles m p 96 97° (Found C 69 6 H 7 2 $C_{17}H_{22}ONCl$ requires C 69 98 H 7 55%) Its *picrate* crystallized from alcohol in yellow needles, m p 130-32°

2 β *Diethylaminoethyl* 3 *methyl* 4 *chloro* 8 *methoxyquinoline* (III R CH_3)

A mixture of II (R CH_3 6 6 g) diethylamine hydrochloride (3 3 g) paraformaldehyde (1 8 g) and 3 drops of concentrated HCl in absolute alcohol (10 cc) was heated on a water bath After an hour a further amount (1 g) of paraformaldehyde was added and heating continued for another 2hr Alcohol was removed on a water bath the residue was dissolved in water basified with caustic soda and the base was extracted with benzene The benzene solution was dried over anh Na_2SO_4 and dry HCl gas was passed in it when the hydrochloride separated It was filtered and washed with dry acetone The substance was highly hygroscopic The *picrate* was crystallized from large volume of alcohol in brown needles m p 195 96° (Found N 13 4 $C_{23}H_{26}O_8N_5Cl$ requires N 13 07%)

H_5)
79° (Found

2 β *Diethylaminoethyl* 3 n *propyl* 4 *chloro* 8 *methoxyquinoline* (III $R-C_3H_7$)

The *picrate* crystallized from alcohol in yellow needles m p 158 60° (Found N 12 6 $C_{25}H_{30}O_8N_5Cl$ requires N 12 43%)

C_4H_9)
lles m p 151 52°

2 β *Diethylaminoethyl* 3 n *amyl* 4 *chloro* 8 *methoxyquinoline* (III R C_5H_{11})

The *picrate* crystallized from alcohol in yellow needles m p 129 30° (Found N 12 1 $C_{27}H_{34}O_8N_5Cl$ requires N 11 83%)

2 β *Diethylaminoethyl* 3 n *hexyl* 4-*chloro* 8 *methoxyquinoline* (III, R C_6H_{13})

The *picrate* crystallized from alcohol in yellow needles m p 139 40° (Found N 11 4 $C_{28}H_{36}O_8N_5Cl$ requires N 11 55%)

2 β *Diethylaminoethyl* 3 *methyl* 4 *chloro*-8 *hydroxyquinoline* (IV, $R-CH_3$)

A mixture of crude II
and water (28 cc) was
to ice partly neutralized
monia under cooling Th
caustic soda On acid fi
compound separated out
The *picrate* crystallized fro
N 13 6 $C_{22}H_{24}O_8N_5Cl$ r

(IV, $R-C_2H_5$)
llow needles m p 190-92°
)7%)

2 β *Diethylaminoethyl* 3 n *propyl*-4-*chloro* 8 *hydroxyquinoline* (IV, R C_3H_7)

The *picrate* crystallized from alcohol in yellow needles m p 170 71° (Found N 12 8 $C_{24}H_{28}O_8N_5Cl$ requires N, 12 74%)

2 β *Diethylaminoethyl* 3 n *butyl* 4 *chloro*-8 *hydroxy quinoline* (IV, $R-C_4H_9$)

The *picrate* crystallized from alcohol in yellow needles m p 115 16° (Found N, 12 3 $C_{25}H_{30}O_8N_5Cl$ requires N 12 43%)

120 to 130° in a Dean and Stark apparatus till no more water collected Benzene was then distilled off and the residual viscous mass was poured into boiling diphenyl oxide After boiling for 15 min, the mass was cooled, diluted with petroleum ether, kept overnight, filtered and washed with fresh petroleum ether

The yield was 55% It was crystallized from alcohol in white needles, m p 289-91° (Found C, 70 5, H, 6 6 $C_{12}H_{13}O_2N$ requires, C, 70 94, H, 6 4%)

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It was prepared from *o*-anisidine and 2 *n* amyl-acetoacetic ester in 55% yield, white needles (alcohol), m p 165° (Found C, 73 9, H, 7 9 $C_{16}H_{21}O_2N$ requires C, 74 13, H, 8 12%)

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2 3 *Dimethyl* 4-*chloro*-8-*methoxyquinoline* (II, $R=CH_3$)

A mixture of I ($R-CH_3$, 15g), $POCl_3$ (35 cc) and PCl_5 (3 g) was heated in an oil-bath at 125-30° for 3 hr The product was then cooled and poured on to ice The aqueous solution was partly neutralized, purified with charcoal and basified with ammonia under cooling The chloro compound separated out as fine needles and was crystallized from petroleum ether in colourless fine needles, m p 132 33°, yield, 14 g (Found C, 64 8, H, 5 3 $C_{12}H_{12}ONCl$ requires C, 65 01, H 5 41%)

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Crystallized from petroleum ether in colourless fine needles, m p 115° (Found C, 66 0, H 5 9 $C_{13}H_{14}ONCl$ requires C, 66 24, H, 5 94%) The *Picrate* crystallized from alcohol in yellow needles, m p 163-65°

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n colourless needles, m p 122 23°

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Picrate crystallized from alcohol in yellow needles, m p 165-66°

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The *Picrate*

2-*Methyl* 3 n-*amyl*-4 *chloro* 8 *methoxyquinoline* (II, $R=C_5H_{11}$)

Crystallized from petroleum ether in colourless needles, m p 101-102°.

(Found C, 68 7, H, 7 0 $C_{18}H_{20}O$ NCl requires C, 69 18, H 7 2%) The *picrate* crystallized from alcohol in yellow needles m p 161 62°

)5%) Its *picrate* crystallized from alcohol in yellow needles, m p 150 52

paraformaldehyde was added and heating continued for another 2hr Alcohol was removed on a water bath the residue was dissolved in water basified with caustic soda and the base was extracted with benzene The benzene solution was dried over anh
chloride separated
was highly hygroscop
in brown needles
N, 13 07%)

2 β *Diethylaminoethyl* 3 *ethyl* 4 *chloro* 8 *methoxyquinoline* (III, R C_2H_5)
The *picrate* crystallized from alcohol in brown needles m p 178 79° (Found N, 13 1, $C_{24}H_{28}O_8N_5Cl$ requires N, 12 74%)

2 β *Diethylaminoethyl* 3 n *propyl* 4 *chloro* 8 *methoxyquinoline* (III R – C_3H_7)
The *picrate* crystallized from alcohol in yellow needles, m p 158 60° (Found N, 12 6 $C_{25}H_{30}O_8N_5Cl$ requires N 12 43%)

C_4H_9)
lles m p 151 52°

2 β *Diethylaminoethyl* 3 n *amyl* 4 *chloro* 8 *methoxyquinoline* (III R – C_5H_{11})
The *picrate* crystallized from alcohol in yellow needles, m p 129 30° (Found N 12 1 $C_{27}H_{34}O_8N_5Cl$ requires N 11 83%)

II, R C_6H_{13})
needles m p 139 40°

2 β *Diethylaminoethyl* 3 *methyl* 4 *chloro* 8 *hydroxyquinoline* (IV, R – CH_3)
A mixture of crude
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The *picrate* crystallized f
N, 13 6 $C_{22}H_{24}O_8N_5Cl$

2 β *Diethylaminoethyl* 3 *ethyl*-4 *chloro* 8-*hydroxyquinoline* (IV, R – C_2H_5)
The *picrate* crystallized from alcohol in yellow needles, m p 190-92° (Found N 13 1 $C_{23}H_{26}O_8N_5Cl$ requires N, 13 07%)

(IV, R – C_3H_7)
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%)

2 β *Diethylaminoethyl* 3 n *butyl* 4 *chloro*-8 *hydroxy quinoline* (IV, R – C_4H_9)
The *picrate* crystallized from alcohol in yellow needles, m p 115 16° (Found N, 12 3 $C_{25}H_{30}O_8N_5Cl$ requires N, 12 43%)

2-β-Diethylaminoethyl-3-n-amyl-4-chloro-8-hydroxyquinoline (IV, $R = C_5H_{11}$):
The *picrate* crystallized from alcohol in yellow needles, m p 125-26°. (Found N, 12 5 $C_{28}H_{32}O_8N_5Cl$ requires, N, 12 12%).

2-β-Diethylaminoethyl-3-n-hexyl-4-chloro 8 hydroxyquinoline (IV, $R = C_6H_{13}$):
The *picrate* crystallized from alcohol in yellow needles, m p. 126-27. (Found N, 12 1. $C_{27}H_{34}O_8N_5Cl$ requires N, 11 82%).

a period of half an hour The mass was left over-night, filtered, triturated with a solution of dilute sodium thiosulphate and ammonia The pasty greenish brown mass was then dissolved in benzene, the benzene solution was dried (Na_2SO_4). The monohydrochloride was prepared by passing dry HCl gas into the benzene solution under cooling It crystallized from a mixture of absolute alcohol and ethyl acetate, m.p 185-86° (Found I, 27 1 $C_{16}H_{21}ON_2Cl_2I$ requires I, 27 9%)

2-β-Diethylaminoethyl-3 ethyl-4-chloro-5 (or 7)*-iodo-8-hydroxyquinoline* (C, $R = C_2H_5$)
The *hydrochloride* crystallized from a mixture of absolute alcohol and ethyl acetate, m p. 184-86°. (Found I, 26 6 $C_{17}H_{23}ON_2Cl_2I$ requires I, 27·08%). The *hydroiodide* crystallized from alcohol, m p 166-68°.

2-β-Diethylaminoethyl-3-n-propyl-4-chloro-5 (or 7)*-iodo-8-hydroxyquinoline* (C, $R = C_3H_7$):
The *hydroiodide* crystallized from absolute alcohol, m p 177-79°. (Found: I, 43 8 $C_{18}H_{25}ON_2ClI_2$ requires I, 44 2%)

2-β-Diethylaminoethyl-3-n-butyl-4 chloro-5 (or 7)*-iodo-8-hydroxyquinoline* (C, $R = C_4H_9$):
The *hydroiodide* crystallized from absolute alcohol, m p 172-73°. (Found: I, 43 05. $C_{19}H_{27}ON_2ClI_2$ requires I, 43 1%)

Summary

Synthesis of 2-β-diethylaminoethyl 3-alkyl-4-chloro-5 (or 7)-iodo-8-hydroxyquinolines having alkyl substitution of methyl, ethyl, *n* propyl, *n* butyl and *n* amyl groups have been effected with a view to observing their amoebicidal activity.

Acknowledgment

The authors are grateful to the University of Calcutta for the grant of special research fund for chemicals

References

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2 Kaushiva, B S (1957), *J Sci Industr Res*, **16C**: 224

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COMPARATIVE MICROBIOLOGICAL STUDIES OF AMOEBICIDES WITH SPECIAL REFERENCE TO THE DERIVATIVES OF PHENANTHROLINE-QUINONE

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The amoebicides in common clinical use to day have been classified by Armengaud and Blanc (1) according to the scheme given in Table I

TABLE I

Classification of the amoebicides

1 "Tissue amoebicides
   Emetine
   Conessine
   Chloroquine
   Preparations with very high iodine content (X ray contrast media)
2 "Contact amoebicides"
   Phenanthroline-quinone (PQ)
   Iodoxyquinoline
   Emetine-bismuth iodide
   Arsenical preparations etc
3 "Medicaments directed against factors promoting amoebic disease"
   Antibiotics

1 To the first group belong those drugs which are active against amoebae situated within the tissues of the host

2. The second group comprises those compounds which for the most part are poorly soluble and which are active within the intestinal lumen and superficially in the mucosa and in lesions thereof.

The third group of substances owe their activity to their ability to influence *the intestinal milieu in a more or less specific way*, especially through their effect on the abnormal flora associated with and favourable to amoebic infection.

This attempt at classification is useful in so far as it emphasises various factors which contribute to the practical efficacy of a given drug. It is mainly based on clinical experience with well known preparations but also depends on a knowledge of their properties gained from experimental work. Therefore, in order to place new, highly active substances into the appropriate place in such a scheme, a consideration of their characteristics and mode of action as indicated by comparative experimental studies with other amoebicides is of value, as also are early clinical results.

The following are some relevant findings with two members of a new synthetic group of amoebicides—the phenanthroline-quinones (2, 6, 9) taken in comparison with data relating to other well known remedies.

The antiparasitic and antibacterial activity of phenanthroline quinone (P.Q.) and its semicarbazone in comparison with that of other amoebicides

Many amoebicides are not only active against *E. histolytica*, but are also clinically useful for the treatment of intestinal disorders due to the presence of flagellates (*Lamblia intestinalis*) or ciliates (*Balantidium coli*).

Emetine and also chloroquine whose activity against amoebae is well known, may in addition be used for the treatment of trematode infection of the liver (*Fasciola hepatica, Chlonorchis sinensis*) (3).

As will be seen from the Table II, the new substance PQ is not only active against flagellates and ciliates (*i.e.*, *Lamblia*, *Balantidium*, *Chilomastix* and also *Trichomonas*) (4, 5) but like emetine and chloroquine it has also been shown by Coudert and Garin (6) *to possess clinical activity against Fasciola hepatica*.

TABLE II

The clinical spectrum of anti-parasitic activity of a number of amoebicides

Amoebicide	Established activity against: Flagellates	Ciliates	Trematodes	Nematodes
Phenanthroline quinone	Lambliasis *Trichomonas intestinalis*	Balantidiasis	Fascioliasis	Mouse *Syphacea obvelata*
Chlortetracycline	*Trichomonas vaginalis*	Balantidiasis		
Chloroquine			Clonorchiasis	
Emetine			Fascioliasis	Trichuriasis
Carbarsone	*Trichomonas vaginalis*	Balantidiasis		

When we come to nematodes, emetine is active against Trichuriasis (7), whereas PQ has been shown experimentally to be active against the mouse pin worm *Syphacea obvelata*. Other animal parasites against which PQ is also active, are *Trichomonas muris* and *T. criceti*.

Thus PQ displays a broader spectrum of anti-parasitic action than other

amoebicides a fact which, although of general interest, does not add significantly to our knowledge of its special activity as an amoebicide

(1) *Amoebicidal action* in vitro

Amoebicidal activity may be assessed in various types of experiment

Amongst others we have used a method by which it is possible to observe the appearance of amoebae microscopically during continued exposure to known concentrations of active substance (9) Living amoebae, together with the

apparently undamaged immobile forms are observable

The amoebicidal potency of various substances can be expressed in terms of the threshold concentration necessary to kill the amoebae In this connection the choice of medium plays an important part

At this stage it is interesting to digress a little to discuss a matter of theoretical interest concerning the role of certain heavy metal ions in the culture medium which may have an important bearing on the mechanism of action Certain constituents such as metal ions may affect the activity of amoebicides in different

zone is increased by the presence of Cu^{++} but reduced by Fe^{++} whereas chlortetracycline for instance is only inhibited by Fe^{++} and not affected by Cu^{++} In the case of the other compounds listed the presence or absence of ions of these two metals does not affect their activity

TABLE III

Influence of Cu and Fe sulphate on the in vitro *activity of various amoebicides*

Amoebicide	Effect of metal ions	
	Cu^{++}	Fe^{++}
PQ Semicarbazone	Synergism	Antagonism
Iodochloroxyquinoline	~	~
Chlortetracycline	~	Antagonism
Carbarsone	~	~
Fumagillin	~	~
Emetine	~	~

It should be pointed out that this effect of metallic ions on amoebicidal

in vitro the following results were obtained from experiments using a standard medium with 10% added serum, but without the addition of metallic salts

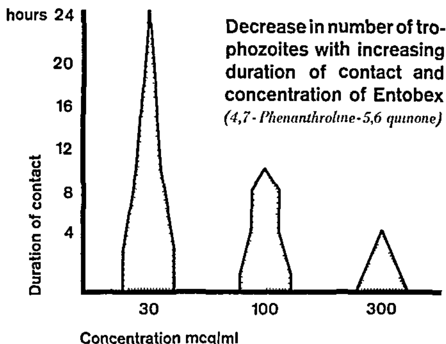


Fig 1 Diminution in the number of mobile amoebae as the duration of contact with various concentrations of amoebicide (PQ) increases (Breadth of columns proportional to percentage of survivors)

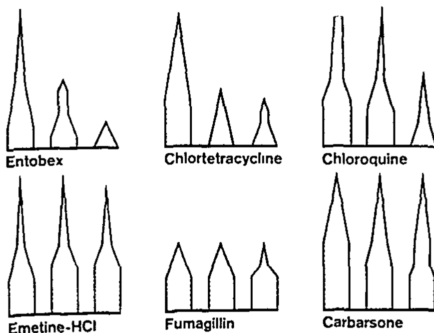


Fig 2 Comparison between amoebicides by reference to their intensity of effect at identical concentrations

Since in the lethal action of an amoebicide the time factor is of importance, it was considered valuable to record the progressive decline in the number of motile forms of amoebae as the duration of exposure to the amoebicide increased (Fig 1)

The comparative potencies of the various amoebicides as indicated by this type of experiment are best represented graphically in Fig 2 Three of the 6 amoebicides namely PQ chlortetracycline and chloroquine differ significantly from the others (emetine fumagillin and carbarsone)

In particular, PQ and chlortetracycline are very similar, the concentration which inactivates all amoebae in 24 hr is in both cases 30 mcg /cc and a threefold increase in concentration leads to an almost identical reduction in the contact time necessary to inactivate all organisms Chloroquine is about 3 times less active than these In the case of emetine the concentration activity relationship is different, and this is also the case for carbarsone both of which substances within the range examined failed to kill amoebae more rapidly as the concentration was increased Fumagillin belongs to the same type, but is more rap d in action than emetine and also the above mentioned preparations at least in the lowest of concentrations tested The concentration activity relationship however, was the same as that for emetine within the range tested

By this method the activity of various substances is more exactly defined than by the simple determination of the minimum effective concentration However, in sp te of the qualitative differences in the mode of action of various p eparations thus indicated there were no corresponding differences detectable in the morphological characteristics during the period leading to the death of the organisms as seen under the microscope This raises the question of whether some of the differences observed might not be explained by an indirect effect through an influence on the accompanying flora

It was therefore of interest to consider this in more detail

(2) *Antibacterial action* in vitro

Lamy and Molinari (10) have shown that the antibiogramme ' of the concomitant flora of various strains of amoebae varies That is to say, the spectrum of sensitivity for a given mixture of concomitant flora towards a number of antibiotics is typical for the specific mixture of organisms associated with a particular strain of amoeba

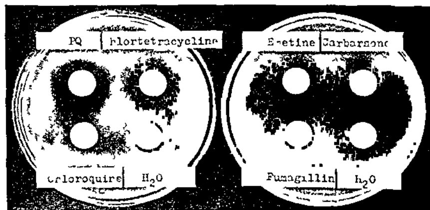


Fig 3 Comparison between amoebicides by reference to their antibacterial activity (zones of growth inhibition in plate tests)

It was, therefore, pertinent to know the antibacterial action of the chosen amoebicides against the concomitant bacteria of our laboratory strain of *E histolytica* We have submitted the six above-mentioned amoebicides to the disc test against the mixed flora concerned, and obtained the results depicted in [illegible]

1% concentration

The antibacterial activity of the first three and the inactivity of the second three is in accordance with the division which we have previously described on the basis of the time-concentration relationship in the direct anti parasitic activity and in [illegible]

from th [illegible]

Th [illegible]

does not adequately characterize certain important chemotherapeutic agents. Thus the pattern of activity against a number of important bacterial species plays a prominent part For example, PQ and chlortetracycline illustrate well this point The latter (Fig 4) shows an ill defined but uniform zone of inhibition

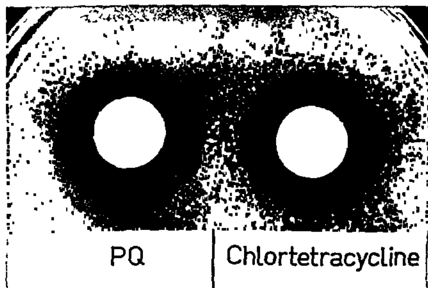


Fig 4 Differentiation between the antibacterial activity of phenanthroline-quinone (PQ) and that of chlortetracycline as evidenced by the zones showing growth inhibition of a mixed flora

[illegible]

experiments

As supplement to Table II giving the spectrum of anti-parasitic action, it may be seen from Table IV that PQ, iodochloroxyquinoline and chlortetracy-

TABLE IV

Antibacterial spectrum of some amoebicides

Preparation	Threshold bacteriostatic concentration (µg/cc) against					
	Staph aureus	*Strept haemolyt*	*Strept faecalis*	*Esch coli*	*Salm typhi*	*Shig sonnei*
Phenanthroline-quinone	5	5	10	25	10	10
Iodochloroxyquinoline	5	5	50	25	50	5
Chlortetracycline	1	0 5	1	5	5	5
Chloroquine	>1000	>1000	>1000	>1000	1000	1000
Emetine	>1000	>1000	>1000	1000	1000	1000
Fumagillin	>100	>100	>100	>100	>100	>100

cline possess an extensive anti bacterial spectrum against various gram positive and gram-negative bacteria (6) As judged by the minimum bacteriostatic concentration chlortetracycline is the most active of this group of compounds In contrast, the so called specific amoebicides emetine, fumagillin and chloroquine are practically inactive also against these bacteria

The question of whether the mode of action of various amoebicides depends essentially on the presence or absence of an antibacterial component, is not only [illegible]

will also differ in their mode of action *in vivo*

However, since in animal experiments other properties such as absorption excretion, tissue concentration etc, are also of importance, the analysis of mechanisms of chemotherapeutic activity is made more difficult

In attempting a comparison *in vivo* of the value of two or more amoebicides the ratio of their minimum therapeutically effective doses is relevant

TABLE V

The minimum effective dose of some amoebicides in experiments on rats

Amoebicide	Effective dose mg/kg *	Amoebicidal effect (%)	Toxicity mg/kg ** (mouse)	Effective dose / Toxic dose
Phenanthroline quinone	20	78	300	0, 07-0, 17
	50	100		
Chlortetracycline	50	86	>2000	<0 05
	100	100		
Chloroquine	100	50-100	250	>0 4
Emetine	5	100	10	0 5
Fumagillin	1	70	*500*	*0 002-0 004*
	2	100		
Carbarsone	250	70	500	>0 5

* Single daily dose p c on 4 consecutive days Assessment on 5th day

** Single daily dose p c on 5 consecutive days Duration of observation 10 days

(3) *Amoebicidal activity in the rat intestine*

The data reproduced in Table V, which were worked out in our laboratories using the method of Jones (1947) are designed to provide a uniform basis for

comparison In this respect, it is of interest that the values obtained for the

takes into account the margin between the minimal therapeutic dose and the toxic dose in the mouse, as shown in Table V, the order of precedence alters somewhat, in as much as emetine becomes one of the last in the list and PQ comes

any attempt

established

ute toxicity,

n vitro, must

possibilities

to be considered as regards the *in vivo* activity of the other amoebicides In

(4) *Effect on the intestinal flora of rats*

Although the task of making a quantitative and qualitative analysis of the

TABLE VI

Effect of amoebicidal doses of PQ, chlortetracycline and emetine on the faecal flora of rats

Amoebicide	Dose* mg /kg	Change in the number of bacteria in the faeces		
		Coliform bacteria	Other aerobic bacteria	Fungi
PQ	50	Increase	Increase	No change
Chlortetracycline	100	(Decrease)	(Decrease)	Increase
Emetine	5	No change	No change	No change

* Daily oral dose given 5 times within 5 days (Where the result is quoted in brackets the probability of error is P=>0 05 Wilcoxon's test)

at all

Data from our laboratories (9) indicate that the intestinal flora of rats reacts to doses of PQ in various phases An initial reduction in the number of bacteria is followed by an increase to levels higher than those determined prior to treatment Although similar alterations in the number of bacteria have also been encountered after treatment with other antibacterial substances it should be noted that PQ has a particularly pronounced effect in this respect and therefore constitutes an exception among amoebicides the practical significance of this peculiarity, however can only be determined in the light of further clinical and laboratory investigation Since it is technically difficult to establish with any certainty, effects exerted upon non specific pathological intestinal flora in man additional experimental research along these lines would seem to be indicated at the same time as studies on man (12) The numerous findings which have emerged from our experiments with the six amoebicides compared here provide renewed evidence for the assumption that the amoebicides differ in various respects as regards their mode of action they also show that the new preparation (PQ) is one which, compared with the ordinary run of amoebicides merits a position in a special category

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INCIDENCE AND EPIDEMIOLOGY OF INTESTINAL PARASITES WITH SPECIAL REFERENCE TO INTESTINAL AMOEBIASIS AND GIARDIASIS

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It is a common experience of clinicians in the tropics and elsewhere that symptoms of vague abdominal pain and discomfort are often wrongly attributed to chronic surgical conditions of the abdominal organs, and lead to an uncalled for surgical interference Less frequently these symptoms and signs very closely mimic those of chronic appendicitis cholecystitis or peptic ulcer, but in fact are due to infestation with one of the intestinal parasites and when the infesta

was prompted by such an observation

The population of a large city was studied under the following groups

Group A It comprised carefully selected apparently healthy individuals, without any abdominal symptoms and was investigated to find out the 'parasitic spectrum' of the so called healthy carriers

Group B This included persons with chronic abdominal ailments

Group C This consisted of cases of acute dysentery These cases were carefully selected with the co operation of the clinicians who referred them to us as early as possible in the course of the disease and before instituting any treatment In addition to the normal routine stool examination these samples were cultured on desoxycholatecitrate agar with a view to isolating pathogenic bacteria that might be responsible for the desentery

Materials and Methods

Materials The results of this survey are based on the results of a single

of parasites

In collecting a fresh sample of faeces the following precautions were observed

(i) It was made certain that the person was not taking any preparation

amination and,
were given ½ oz
prior to their
discarded As
a rule second or third sample of faeces, after the purgative had started to act, gave us the desired material

(*iii*) The stool was collected in a sterile, clean, dry glass Petri-dish free from antiseptics, soap or other detergents The person under investigation was instructed not to pass urine in the Petri dish as it has a lethal effect on the trophozoite forms of the protozoa (1)

Method of preparing the slide for examination As recommended by Craig (2) a small portion of faeces was thoroughly mixed with a drop of normal saline, placed on a clean slide, carefully emulsified and a cover slip placed on it Care was taken not to make too thick emulsions, and as a guide, it was made sure that newsprints could easily be seen through it This procedure was also repeated with a drop of Lugol s iodine If blood or mucus was present in the faeces, small portions of these were examined as well

The *sample of faeces was concentrated by the zinc sulphate centrifugal* flotation method of Faust *et al* (3) and the float examined with a drop of Lugol's iodine

With properly adjusted sub stage and iris diaphragm (to secure maximum definition of the trophozoite forms of the parasites) these preparations were examined by 10X ocular and 16 mm and 4 mm objectives

Criteria for the identification of the trophozoite forms of E histolytica For the correct diagnosis we are taught to ask two questions Does it move ? Does it contain red blood cells as inclusions ? This is certainly true in cases of acute

E histolytica very often does not contain red blood cells We satisfied ourselves on the following points, before any structure that was changing its shape was labelled as *E histolytica*

(*i*) Its sharper outline in contrast to coarser appearance of other amoebae

(*ii*) Well differentiated, clear, glass like ectoplasm, constituting more than one third of the whole organism, in contrast to the dull, opaque, small and indistinct zone of other amoebae

(*iii*) Its characteristic progressive motility achieved by the formation of a long finger like pseudopodia into which the granular endoplasm flows in a slow but deliberate manner According to Craig (2) even in the absence of red blood cells as inclusions one is justified in making a diagnosis of *E histolytica*, if this characteristic formation of pseudopodia and the flow of endoplasm is present

It acute dysenteric cases, in addition to these, the following features were frequently seen

(*i*) Presence of red blood cells as inclusions

(*ii*) Marked directional motility, amoeba traversing long distances under the microscope

(*iii*) *It assumes a slung like form and advances with a definite polarity—the* advancing end being rounded and permanent, while the trailing end is more pointed and often a mass of granular debris is adherent to it

In view of the findings of Meleney and Zuckerman (6) that small races became large on culture media, the size of the protozoa has no diagnostic value

Results

In this paper stress is laid on the incidence of the parasites and their association with signs and symptoms

The number of cases investigated under the groups A, B and C were from the following sources

	Sources	*Group A*	*Group B*	*Group C*
1.	Hospital cases	44	690	100
2	Members of nursing staff	57	44	
3.	Factory hands	183	96	4
4	Inmates of a reformatory school	100	180	
	Total	384	1,010	104

The actual incidence of the various intestinal parasites in these groups is shown in Tables I and II, and their percentages in Figs 1 and 4 Fig 1 shows that *E histolytica* and *G lamblia* occur much more frequently in the faeces of group B than in those of group A The X^2 test shows that these differences are significant The value of *P* (probability occurrence by chance alone) being less than 0 001 in both the cases The incidence of symptoms and signs where these two parasites occurred either as a single or a combined infection, has been worked out in detail and is presented in Figs 2 and 3 Table II also shows the physical findings and therapeutic response in group C

TABLE I

Showing incidence of intestinal parasites

No	Parasite	Form	Group A (384) No		Group A (384) Percent	Group B (1, 010) No		Group B (1, 010) Percent
1	*E histolytica*	Trophozoite	38			237		
		Cyst	26	= 77	20 1	62	=359	35 6
		Both	13			60		
2	*Endamoeba coli*	Trophozoite	111			232		
		Cyst	19	=189	49 2	59	=425	42 1
		Both	59			134		
3	*Endolimax nana*	Trophozoite		36			88	
4	*Iodamoeba butschlii*	Cyst		3	10 9		11	10 2
5	*D entamoeba fragilis*	Trophoziote		1			4	
6	*Giardia lamblia*	Trophozoite	25			134		
		Cyst	17	= 44	11 5	55	=217	21 5
		Both	2			28		
7	*Trichomonas hominis*	Trophozoite		32			95	
8	*Ch lomastix mesnili*	Trophozoite						
		Cyst	4	— 6	9 9	8	= 20	2 0
		Both	2			12		
9	Hookworm	Ova		47	12 2		168	16·6
10	*Ascaris lumbricoides*	Ova		86	22 4		204	20 2
11	*Trichuris trichiura*	Ova		62	16 2		172	17·0
1	*Strongyloides stercoralis*	Larva		3	0 8		6	0 6
13	*Enterobius vermicularis*	Ova		10	2 6		20	2 0
14	*Hymenolepis nana*	Ova		10	2 6		25	2 5
15	*Taenia (saginata and solium)*	Ova		1	0 3		9	0 9
16	*Blastocystis hominis*			65	16 9		154	15 3
17	Yeast			40	10 4		66	6 5
18	Sarcinae			96	25·0		178	17·6

TABLE II

Intestinal parasites in human population Group C—104 cases of acute dysentery

Pathogen	Total no	Per cent	Pathogen demonstrated	Presumptive diagnosis	Pathogenic bacteria: Flexner G I	Flexner G II	*Proteus morgan*	*E typhi*	*B pyocyneous*	*E coli*	*G lamblia*	Other parasites found: *Ch mesnili*	*T hominis*	Hookworm	*Strongyloid*	*Ascaris*	*Trichuris*	Other characters of the stool: Charcot Leyden cyrstals	R B C clumped	R B C movement	Reaction of stool: Acid	Alkaline	Assoc of alcoholism with liver findings	History of previous dysentery	Response to treatment
1 Amoebic infection only	46	44 2	46							3	12	3	6	3	1	6	1	3	9	3	6	41	10	26	All responded to emetine hydrochloride, ⅓ gr sub cut once a day for 7 days One case (a medical student) relapsed repeatedly and ended up with ulcerative colitis which responded only to small repeated blood transfusions At the end of treatment seven were still passing cysts though asymptomatic Out of 17 people with liver findings 12 showed improvement
2 Amoebic and bacillary mixed infection	5	4 8	3	2			1	1	1				1					1				5		4	All except one (typhoid carrier) needed combined therapy of emetine as in (1) and sulphaguanidine as in (3) (*a* and *b*) The typhoid carrier with coincident amoebic dysentery responded to emetine alone
3 Bacillary dysentery	39	37 5	(*a*) 12		6	3	4							1								12		6	(*a*) & (*b*) —Responded strikingly (48—72 hr) to sulphaguani-

TABLE II (*Contd*)

Pathogen	Total no	Per cent	Pathogen demonstrated	Presumptive diagnosis	Pathogenic bacteria: Flexner G I	Flexner G II	*Proteus morgan*	*E typhi*	*B pyocyaneus*	*E coli*	*G lamblia*	Other parasites found: *Ch mesnili*	*T hominis*	Hookworm	*Strongyloid*	*Ascaris*	*Trichuris*	Other characters of the stool: *Charcot Leyden crystals*	R B C clumped	R B C movement	Reaction of stool: Acid	Alkaline	Assoc of alcoholism with liver findings	History of previous dysentery	Response to treatment
																									dine by mouth 6 g initial dose and 3g every 4 hr till stool became less than 5 per day then 3 g every 8 hr till stool was normal In (*a*) stool cultures at the end of treatment were negative except in one case of mixed infection (*Flex* G II and *Proteus morgan*) in which *Flex* G II was again grown a potential carrier
			(*b*)	12												2	1				2	10		6	(*c*) These were clinically chronic and mild cases No striking response to chemotherapy (3gr of sulphaguanidine every 8 hr) Five responded partially but relapsed Ten were given emetine hydrochloride, ½ gr sub cut daily for 4 days without any change Stool examination repeated *without success to isolate* organisms Bleeding piles excluded Being ambulatory cases could not be induced to come for further investigation or treatment
			( )	15																		15		6	

TABLE II (Contd)

Pathogen	Total no	Per cent	Pathogen demonstrated	Presumptive diagnosis	Pathogenic bacteria: Flexner G I	Flexner G II	*Proteus morgan*	*E typhi*	*B pyocyaneus*	*E coli*	*G lamblia*	Other parasites found: *Ch mesnili*	*T hominis*	Hookworm	*Strongyloid*	*Ascaris*	*Trichuris*	Other characters of the stool: Charcot Leyden cyrstals	R B C clumped	R B C movement	Reaction of stool: Acid	Alkaline	Assoc of alcoholism with liver findings	History of previous dysentery	Response to treatment
4 *Giardia lamblia*	8	77	8								8		2				1	1			2	6		4	In all these colic and tennesmus was less marked, abdominal pain was more vague, and the stools contained less blood but more mucus 0 1 gr mepacrine t d s daily by mouth for five days was given Six improved and at the end of treatment had no dysenteric symptoms nor the parasite in the stools Two showed slight improvement and the infection persisted In the cured series, in one leucorrhoea persisted but in another the associated urticaria vanished
5 Hookworm infec-	2	19	2											2							.	2		1	Hexyl resorcinol, 1 gr, in capsule, on empty stomach followed by a *salinepurge 21 1ı afterwards* Dysenteric symptoms disappeared in both the cases though pulmonary symptoms persisted Ova detected in the stool of one of *them even after* treatment

TABLE II (*Contd*)

Pathogen	Total no	Per cent	Pathogen demonstrated	Presumptive diagnosis	Pathogenic bacteria: Flexner G I	Flexner G II	*Proteus morgan*	*E typhi*	*B pyocyaneus*	*E coli*	*G lamblia*	Other parasites found: *Ch mesnili*	*T hominis*	Hookworm	*Strongyloid*	*Ascaris*	*Trichuris*	Other characters of the stool: Charcot Leyden crystals	R B C clumped	R B C movement	Reaction of stool: Acid	Alkaline	Assoc of alcoholism with liver findings	History of previous dysentery	Response to treatment
6 *Strongyloides stercoralis*	1	0 9	1												1							1			Not treated
7 *Ascaris lumbricoides*	3	2 9	3													3	1				1	2			Two cases were treated The third refused treatment Hexyl resorcinol 1 gr on empty stomach followed by a saline purge an hour afterwards One responded well, passed several adult worms and was asymptomatic by third day, second was partially relieved Stool of both continued to show ova Pulmonary symptoms not relieved

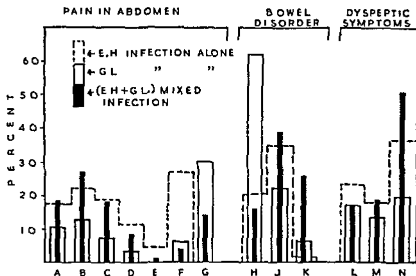


Fig 3

A Pain in epigastrium B Pain in Rt iliac fossa C Pain in Lt iliac fossa D Pain in Rt hypochondrium E Pain in Lt F Pain in umbilical region G Vague pain all over H Diarrhoea J Constipation K Alternate constipation & diarrhoea L Anorexia M Nausea & vomiting N Flatulence

Discussion

Most of the surveys of intestinal parasites done in recent years in different localities have been of a temporary floating population, *e g*, troops in various theatres of war or temporary army hospitals etc This survey is of significance as it deals with more or less static population of a city

Group A The incidence of various parasites in this group shows that highly

discussion A few important parasites, however, have been picked out by several workers for such a study *E histolytica* has been more frequently investigated than any other

such a group is shown in Table IV

Group B Fig 1 reveals a few interesting features in the infection rate of groups A and B In people with abdominal symptoms there is an appreciably higher rate of infestation with *E histolytica*, *G lamblia* and hookworm than in those who are symptom-free, the differences are 15 5, 10 0 and 4 4%, respectively

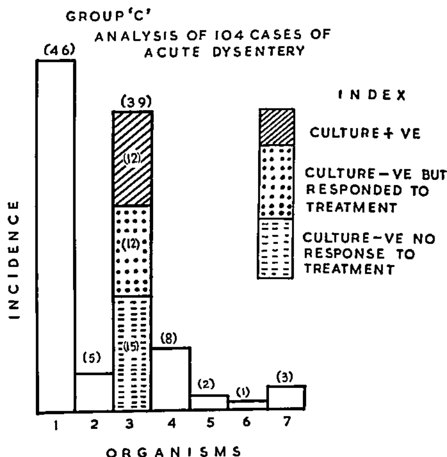


Fig 4

1 *E histolytica* 2 *E histolytica*+Bacillary 3 Bacillary infection 4 *Giardia lamblia*
5 Hookworm 6 *Strongyloid stercoralis* 7 *Ascaris lumbricoides*

E coli and round-worm infection is lower in group B by 7 6 and 2 2%, respectively. Other protozoa and helminths are more or less at the same level and non-pathogens, such as *Blastocystis hominis*, yeast and sarcinae are less common in group B

The incidence of *E histolytica* infection in people with vague abdominal

symptoms 30 to 35 % are infested

The incidence of *G lamblia* infestation in group B is nearly double to that in group A, and in our opinion it is an important parasite in the production of chronic and vague abdominal symptoms In Asmara, Sofia and Ciaravino (15) found only 2 1% people with abdominal symptoms to be infested with this parasite

In contrast to the high incidence of tapeworms (especially the *Tenia* group) infestation in the human population of other countries, their incidence in this

TABLE III

No	Investigator (Ref)	Locality	No of cases examined	No positive	Percentage
1	MacAdam (7)	India	351	46	13.0
2	Hardy and Spector (8)	Chicago	161	25	15.5
3	Cannan (9)	Jerusalem	180	40	20.2
4	Patel (10)	Bombay	125	54	43.3
5	Leitman and Vitlinskaya (11)	Tashkent (USSR)	1 002	128	12.7
6	Shrivastav (12)	Bombay	384	77	20.2

TABLE IV

No	Investigator (Ref)	No of healthy adults investigated	Percentage
1	Kofoid quoted by Craig and Faust (14)	2 300	5.7
2	Jepps quoted by Craig and Faust (14)	971	13.2
3	Leitman and Vitlinskaya (11)	1 002	13.9
4	Shrivastav (12)	384	11.5

TABLE V

No	Investigator (Ref)	Locality	Total no examined	No found positive	Percentage
1	Hardy and Spector (8)	Chicago	33	14	42.0
2	Cannan (9)	Jerusalem	400	122	30.5
3	Sofia and Ciaravino (15)	Asmara	700	209	29.8
4	Liebermann (16)	Natal	105	31	29.5
5	Brown (17)	N Africa & European theatres	298	65	21.8
6	Patel (10)	Bombay	101	58	58.0
7	Shrivastav (12)	Bombay	1 010	359	35.6

survey, in any group, is remarkably low but this can be easily explained. The Hindus and Muslims, who constitute the majority of the population in the city, on religious grounds do not eat either beef or pork respectively. The few cases of *Tenia* infection detected were mostly in the Christian population of the city. Among the tapeworms detected *H nana* infestation was the commonest.

Association of parasites with signs and symptoms in group B *E histolytica* and *G lamblia* had the highest incidence among the pathogenic parasites in this group Their association with the signs and symptoms as shown in Figs 2 and 3, may briefly be summarized in order of their frequency (Table VI)

It is clear from this summary that the symptom complex and signs in these two infections are remarkably different in order of frequency and in details when

TABLE VI

Parasite	Symptoms and signs in order of frequency	Details of symptoms and signs in order of frequency
E histolytica alone	1 Pain (73%)	Right iliac fossa and round about umbilicus
	2 Dyspepsia (56 9%)	Flatulence and anorexia
	3 Disorder of bowel (55%)	Constipation
	4 Constitutional symptoms (36 1%)	Loss of weight
Signs	1 Involvement of caecum (47 3%)	Tender and palpable
	2 Involvement of liver (17 1%)	Chronic appendicitis (5 4%)
	Common surgical condition misdiagnosed	Chronic peptic ulcer (5 0%)
G lamblia alone	1 Bowel disorder (89 8%)	Frequency of motions (61 8%)
	2 Pain (63 5%)	Vague pain all over the abdomen and epigastrium
	3 Dyspepsia (43 2%)	Flatulence and anorexia
	4 Constitutional symptoms (24 8%)	Fever
Signs	1 Involvement of caecum (31 4%)	Only tender
	2 Involvement of liver (8 0%)	Chronic peptic ulcer (3 4%)
	Common surgical conditions diagnosed	Chronic appendicitis (2 5%)
E histolytica and *G lamblia* together	1 Bowel disorder (80 8%)	Constipation with alternate diarrhoea
	2 Pain (72 7%)	Iliac fossa and epigastrium
	3 Dyspepsia (65 7%)	Flatulence and nausea and vomits
	4 Constitutional (28 3%)	Fever and loss of weight
Signs	1 Involvement of caecum (51 5%)	Palpable and tender
	2 Involvement of liver (17 2%)	Chronic appendicitis (5 0%)
	*Common surgical conditions mis*diagnosed	Peptic ulcer (3 0%)

abd men and n the ep ga tr m fl len a

Fraser and Taylor (19) and Monat and McKinney (20) found frequent occurrence of diarrhoea in *G. lamblia* infection, but did not find vague pain all over the abdomen or in epigastrium as a predominant symptom. The latter

The relative incidence of amoebic and bacillary dysentery has always been a

observations have been contrary to this belief, and he found amoebic dysentery to be one and half times as common as the bacillary form. Our figures lean towards Payne's observations and it appears that the pendulum has now started to swing in the direction of amoebic dysentery again (Table VII).

TABLE VII

Investigator (Ref.)	Locality	Percentage of amoebic	Percentage of bacillary	Percentage of of a mixed infection
1 Cunningham (27)	East Bengal	14.0	86.0	
2 Vaidya (28)	Bombay	35.0	65.0	
3 Payne (21)	East India	50.7	31.6	6.0
4 Shrivastav (12)	Bombay	44.2	37.5	4.8

When comparing the relative incidence of amoebic and bacillary dysentery an important point, which may be mentioned here, is often omitted. When dysentery assumes epidemic proportions, a comparison between the two is not justifiable, because depending on the nature of the epidemic one or the other type

will naturally predominate What is more important is to find-out the endemic prevalence of the two dysenteries in a static population, and that has been the attempt in this survey During the period that this survey was carried out there was no recognizable epidemic or either dysentery and, therefore, the figures give their endemic incidence in the population

The cases included under the bacillary type in the survey need a word of explanation The criteria for the diagnosis of bacillary dysentery had been much wider and less strict than for the diagnosis of amoebic type For amoebic dysentery the demonstration of actively motile trophozoite stage of *E histolytica* was an essential feature, while for the diagnosis of bacillary dysentery cultural findings were not the only criterion, the cellular picture of the faecal exudate,

he cases
en such
the last

Veghelyi quoted by Strong (36) found that out of 144 children infected with this parasite one fourth showed blood and mucus in stools Brumpt, quoted by Strong (36) reported a dysenteric syndrome resembling amoebic dysentery but not ameliorated by emetine Our findings are in agreement with the later group of workers

Among helminths causing acute dysentery, Fleming (37) and Roger and Dammin (38) found only intractable diarrhoea, while Sangster (39) found 25 cases with blood and mucus in the stool, associated with hookworm infection *S stercoralis* infection causes not only chronic diarrhoea, but also acute dysentery Levin quoted by Strong (36) in a report of 92 cases, noted mucus and blood in the stools of 5 cases, while Hinman (40) found 14 cases with acute dysentery in a study of 85 cases Role of *A lumbricoides* in causation of acute dysentery is interest- [illegible] reported, though Faust quoted
off small masses of intestinal
uch cases one responded to
hile the third could not be
treated

We did not come across any *Balantidium coli* or *Isospora hominis* infection in this investigation

The association of chronic alcoholism and presence of hepatic signs and symptoms in cases of acute amoebic dysentery is another interesting feature of this group Da Silva (41) in 200 cases of amoebic hepatitis in Ceylon, found such

an association in 176 cases, while in our series, out of 46 cases, 17 had hepatic symptoms, in which 10 were alcoholics. This association raises three possibilities.

(1) It may be a mere coincidental finding

(2) Chronic alcoholism may be responsible for early cirrhotic change in the liver, but in our series, there were no other signs of cirrhosis, in form of ascites or oedema of scrotum or limbs.

(3) Chronic alcoholism lowers the resistance of the body as a whole and of liver in particular and amoebic involvement of liver occurs with ease

This question, however, needs further investigation

What diagnostic significance can be attached to the reaction of the stool in amoebic dysentery is difficult to say, because out of 47 samples only 6 were acidic, while 41 were alkaline to the ordinary litmus paper To our surprise, two bacillary stools also gave an acidic reaction Acton and Knowles (26) described clumping of red blood cells and amoeboid movement of the isolated red cells (due to the movement of adherent spirochaetes to it) in acute amoebic stool In 46 cases of acute amoebic dysentery, clumping of red cells in nine cases and slight amoeboid movement of the outline of the red cells in three cases was found

SUMMARY

20 2%

in this survey.

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A STUDY OF TRYPTIC ACTIVITY AND CHANGES IN BLOOD CONSTITUENTS IN CASES OF INTESTINAL INFESTATION

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It is now well known that malnutrition is a predisposing factor in chronic intestinal infestation Chandler (1) observed that malnutrition pregnancy, old age etc accentuate the disease Faust (2) explained the anaemia observed in hook worm infestation as due to deficiency in protein intake (96% of the haemoglobin molecule is derived from dietary proteins) There are two other theories for the manifestation of anaemia in hook worm disease One takes into account the blood sucking habit of the worm while the other postulates the existence of toxic factors in the worm which depress the bone marrow However, no correlation has been observed between the number of worms present in an individual and the drop in haemoglobin percentage nor has any toxic factor been isolated from the worm so far In this investigation 40 cases of ankylostomiasis have been studied Ten were severe cases in whom haemoglobin was less than 5 g % Twentyfive cases were of moderate severity while the rest were mild in nature In all cases routine blood examinations and serum protein estimations were carried out and the tryptic activity of the duodenal juice was determined

RESULTS

This study showed that as the disease due to hook worm progressed serum proteins continued to decrease Anaemia was a constant feature of severe cases which corresponded to the fall in serum proteins Chandler (1) and Faust (2)

TABLE I

	Protein	Fat	Carbo-hydrate	Iron	Vit A	Calories
Mild cases	85 7	8 2	472	36 4	419	2275
Moderate cases	72 8	6 2	370	31 5	370	1810
Severe cases	56 6	4 9	293	27 7	304	1379

[illegible]

[illegible] An easy explanation becomes available if one compares the tryptic activity of duodenal juice in the three groups (Table II)

TABLE II

	Haemoglobin (g)	Serum protein (g %)	Tryptic activity (units)
Mild	12 0	7 1	14
Moderate	8 8	6 5	9 3
Severe	3 0	6 0	6 5

It will be seen that the fall in tryptic activity corresponds to the fall in haemoglobin and serum protein levels One can, therefore, surmise from the evidence given above that there is some factor in hook worm which depresses tryptic activity and thus cuts off protein intake of the body internally Symptoms of vitamin deficiency seen in these cases are also attributable to the same cause

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THE ACTION OF DRUGS *IN VITRO* ON TAPEWORM (*HYMENOLEPIS NANA*)

A B SEN AND F HAWKING

From the Central Drug Research In t tute Lucknow

This paper describes an investigation of the action of well known chemo therapeutic compounds upon the tapeworm *Hymenolepis nana in vitro* It was hoped that the results obtained would indicate the type of compound to which tapeworms would be susceptible

Materials and Methods

The worm used was *Hymenolepis nana* a common tapeworm of mice, which can be transmitted directly by ova from mouse to mouse since both stages of the worm (cysticercoid and tapeworm) take place in the same animal The strain employed was obtained from the National Institute for Medical Research U K The technique for investigating this worm in the laboratory consists of two parts

(*i*) *Maintenance of the infection in mice*

This technique was based on that of Steward (1) Ova were obtained from mature segments of worms collected from infected mice They are allowed to stand in water for 20 (or 44) hr at room temperature (about 30°C) An appropriate number (500—2000) is given to mice by syringe and stomach tube Under good conditions the percentage of mice which later contained worms was about 80 and the number of worms was 1 to 56 If only one worm was present, 21 days after infection, it was usually a large one about 5 cm long If many worms were present they were small 0 5 1 0 cm

by inserting the needle of a syringe into the upper end and perfusing it with saline If an isotonic solution buffered to be slightly acid (*p*H 6 8) was employed the worms seemed to come out more readily

(*ii*) *Exposure of worms to compounds* in vitro

The worms recovered from the intestine was picked up free from mucus They were then placed per cc and strepto mperature (30 34°C) same amount of anti- Kjeldahl s flasks of 10 cc volume were used because they happened to be available and they proved convenient Solutions of penicillin as high as 2000 units per cc and of streptomycin as high as 1 0 mg /cc were harmless to the worms

One or two worms were placed in each flask which was then bunged Suitable concentrations of the various compounds had been previously added to

nutrient broth in each flask Since it was desired to make a broad survey of a large number of compounds, concentrations were chosen in multiples of 10 The flasks were incubated at 37°C For examination they were placed in a horizontal position under a binocular dissecting microscope with low magnifications where the movements of the worms could easily be observed The worms of the control flasks with drug free medium remained actively motile for 5 days

The action of the drug however, was read after 24 hr because it is unlikely that a drug given by mouth would stay in the intestine longer than this Bacterial growth in the flask did not occur to any appreciable extent

Results

[illegible]

may be questioned whether they might not prove as active as mepacrine in clinical practice

TABLE I

Effect of drugs on H nana in vitro

No	Compounds	1 1 000	1 10 000	1 100 000	1 1 million	1 10 million
1	Ext fil cis	A	A	A	N A	
2	Stilbamidine	A	N A	N A	N A	
3	Arecoline acetarsol	A	A	A	N A	
4	Pentaquin	A	A	A	N A	
5	Pamaquin	A	A	A	N.A	
6	Mel B	A	N A	N A	N A	
7	Dicestal (2 2 Dihydroxy 5 5 -dichlorodiphenylmethane)	A	A	A	A	N A
8	Copper sulphate	A	N A	N A	N A	
9	Tetrachlorethylene	A	A	N A	N.A	
10	Phenothiazine	A	N A	N A	N.A	
11	Mepacrine	A	A	A	N.A	
12	Chloroquine	A	A	A	N.A	
13	Arsenamide	A	A	N A	N A	
14	Santonin	A	N A	N.A		
15	Sulphadiazine	N A	N A			
16	Chloramphenicol	N A	N A			
17	Tetracycline	N A	N.A			
18	Vioform	A	A	A	N A	
19	Carbon tetrachloride	A	A	N A	N A	
20	Camoform	A	N A	N A		
21	Hetrazan	N A	N A			
22	Tartar Emetic	A	A	A	A	N A
23	Miracil D	A	A	A	N.A	
24	Hexylresorcinol	A	A	A	N.A	
25	Piperazine	A	N.A	N A		

A—Active N A —No Action

Confirmation by in vivo *tests*

To confirm whether this *in vitro* method of testing compounds is dependable or not we carried out some *in vivo* tests with some of the more well known compounds Large number of compounds could not be tested because of non-

uniformity of the percentage of "take" in the experimental mice. This was probably because of immunity possessed by the Institute mice as we have not as yet been able to establish a Helminth free' colony of mice.

The general principles followed in these tests are those of Steward's(1). A number of just weaned mice were taken and fed with 500 mature ova (kept in boiled and cooled tapwater for 20 hr at 29°C) of *Hymenolepis nana* with morphine hydrochloride, 0.05 mg/mouse by a stomach tube as stated earlier. These animals were put in groups of five. On the 19th day random sample of faeces were taken from different groups and examined for presence of ova. Only positive groups were taken for experimental purpose. A group of five were left untreated as control. The rest were fasted for 24 hr on the 20th day and treated the next day. All including the controls were fasted for 24 hr before they were sacrificed on the 3rd day after treatment. Small intestine from each

TABLE II

No	Drug	Dose mg/20 g	No. negative / No. treated	Score	Remarks
1	Mepacrine	6	4/5	0.4	Very active
2	Ext. filicis	15	4/5	1.1	Active
3	D cestal	20	5/5	0	Very active
4	Arecoline acetarsol	0.4	3/5	3.2	Active
5	Tetrachlorethylene	0.03 cc	3/5	4.2	Slightly active
6	Hexylresorcinol	10	4/5	1.7	Active
7	Control	—	0/5	32.2	—

Results shown above agree reasonably well with those which we got in *in vitro* tests.

numbered mouse was removed in a straight piece and washed with the help of a 20 cc syringe and blunt needle. The washing fluid used was an isotonic solution buffered to *p*H 6.8. The number of worms were counted and placed in 5 different groups for scoring and the score entered in book. The intestine was then placed in between two glass plates and viewed under a dissecting microscope to find out any worm left over. Scores allocated were as follows:

Very large 20 actual size 1½"
Large 10 actual size about 1"
Medium 5 actual size about ½"
Small ½ actual size about ¼"
Very small 1/10 actual size 1/8"

Discussion

In searching for new chemotherapeutic agents there is always the question whether to use *in vitro* or *in vivo* tests. Generally *in vitro* tests are much simpler but *in vivo* tests are more reliable. When the parasites are hidden away in the tissues, *e.g.*, trypanosomes or filarial worms *in vitro* tests are so unreliable that they hardly are worth doing. On the other hand when the parasites are worms lying in the lumen of the intestine *in vitro* tests seem quite justified since the conditions in the intestine are fairly similar to those in the test tube. Drugs given by mouth reach the intestine with little or no possibility of chemical change—they act upon the worm for a short time—and then they are removed by passage down the intestine for absorption. The technique which has been described for testing drugs on mouse tapeworms has the advantages of simplicity and speed. Many different drugs and concentrations can be tested in a single experiment on the

worms taken from 5 6 mice The results are obtained in 24 hr On minute quantities of the compound are required for the test And there is need to carry out preliminary determinations of the toxicity for mice before t tests are made on the worms Accordingly, the technique is well suited f a screening test in which large numbers of unknown chemical compounds a examined to pick out the few individuals which deserve more detailed stud We plan to apply the same technique to other intestinal worms such as *Nipp trongylus* and *Ancylostoma caninum* It could also be easily applied to any oth intestinal worms for which a source of supply can be arranged

When a large number of compounds have been examined in this way t next step will be to make a detailed study of the selected few compounds whi show a favourable ratio between their activity on the one hand and their toxic on the other The first step will be to test their activity *in vivo*, *i e*, their pow to kill the worms in mice These tests are laborious to carry out and they requ

And then we should hope to arrange clinical trials which are the final criteri whether or not a new compound will be of real practical importance

Acknowledgment

We wish to express our thanks to Dr B Mukerji, Director, Central Dr Research Institute, Lucknow for the facilities provided, and for his helpful c ticism and continued interest in the work

Reference

1 Steward, J S (1955), *Parasit* 45 255

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STUDIES ON THE ANTIHELMINTHIC ACTIVITIES OF SEEDS OF *CARICA PAPAYA* LINN

M K KRISHNAKUMARI AND S K MAJUMDER

From the Central Food Technological Research Institute Mysore

In many tropical countries papaya seed is administered as an anthelmintic In Central and South America the seed [illegible] gue (1) In some parts of India, th [illegible] expelling roundworms Recent *in viv* [illegible] papaya seeds by Robinson (2) and F [illegible] results Chemical studies by Merck (4) [illegible] carpaine ($C_{14}H_{25}O_2N$), is present in [illegible] alkaloid has also been isolated from le [illegible] that carpaine is a potent amoebicide Another alkaloid called carpasemine has also been isolated from papaya seed (7)

Papaya seed, although being widely used as a herbal drug for its ascarifuge action, no experimental evidence, is however, available on its *in vitro* activities on helminths

There h[illegible] [illegible] cultures

In the present communication, the results of *in vitro* studies on the ascaricidal activities of *C papaya* seeds on *Toxascaris transfuga*, *Ascaris* sp and *Ascaris lumbricoides*

worms taken from 5 6 mice The results are obtained in 24 hr minute quantities of the compound are required for the test And there need to carry out preliminary determinations of the toxicity for mice befo tests are made on the worms Accordingly, the technique is well suit a screening test in which large numbers of unknown chemical compoun examined to pick out the few individuals which deserve more detailed We plan to apply the same technique to other intestinal worms such as *J trongylus* and *Ancylostoma caninum* It could also be easily applied to any intestinal worms for which a source of supply can be arranged

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ACKNOWLEDGMENT

We wish to express our thanks to Dr B Mukerji Director, Central Research Institute Lucknow for the facilities provided and for his helpfi ticism and continued interest in the work

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M K KRISHNAKUMARI AND S K MAJUMDER

From the Central Food Technological Research Institute Mysore

papaya seeds by Robinson (2) and Fernando (3) have given little convincing results Chemical s
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also been isolated from papaya seed (7)

Papaya seed although being widely used as a herbal drug for its ascarifuge action no experimental evidence, is however available on its *in vitro* activities on helminths

There have been many attempts to maintain the nematodes outside the host in artificial cultural medium Cavier and Savel (8) have studied the survival time of adult ascaris in a mixture of sodium chloride and sodium carbonate and also in various complex media like Ringer s and Tyrode s solutions Robinson and Gade (9) have suggested the use of Baldwin's solution for worms for *in vitro* cultures

In the present communication the results of *in vitro* studies on the ascaricidal activities of *C papaya* seeds on *Toxascaris transfuga* *Ascaris* sp and *Ascaris lumbricoides*

var *suis* are reported Chopra *et al* (10), Sollman (11) and others (12) have tested the action of thymol and other anthelmintics on earthworms Due to the non availability of adequate numbers of parasitic test worms for trials on the solubility of the vermicidal agent of the seed in some solvents and studies on the stability of the drug-action on storage, earthworm (*Pheretima* sp) was used as test organism in these experiments

Experimental

Collection Worms were obtained from the faeces of the animals in the local zoo garden which were expelled without the administration of any ascarifuge Collection of worms was also carried out at the slaughter house The live ones were immediately transferred to the cultural medium and was maintained at 35°C to 40°C in a vacuum flask for avoiding death due to sudden change in environmental temperature after expulsion and during transport to the laboratory Earthworms were maintained in the laboratory on leaf mould and soil mixture

Selection of medium For the selection of a suitable medium for maintaining worms, preliminary studies were carried out with the following culture media

1) Tyrode's solution
2) Ringer's Solution
3) Baldwin s solution
4) Ringer-Locke solution

On the basis of the survival time and the activity of the worms in the above mentioned media, Ringer-Locke solution was found to be the best This was selected for the experiments The composition was as follows

Modified Ringer Locke solution

Sodium chloride	9 00 g
Potassium chloride	0 42 g
Calcium chloride	0 24 g
Sodium bicarbonate	0 20 g
Glucose	1 00 g
Distilled water	1000 cc

Seeds Dried papaya (honeydew variety) seeds were obtained from a local farm and stored in sealed bottles in a refrigerator These were powdered in a grinder and sieved through 100 mesh whenever required for the trials Water

Toxascaris transfuga, from
is *Ascaris* sp , from polar
e of the solution and an
incubation temperature of 34°C *Ascaris lumbricoides* var *suis* from pigs, lived for 11 days at 34°C while *Pheretima* sp could be maintained for 8 days at 28 29°C in the cultural medium

results of the experiment are presented in Table 4

TABLE I

Comparative in vitro *response of test worms to papaya seed powder and piperazine citrate*

Test worm	Drug	Dosage Drug (g) Ringer Locke (cc)	Death time (hr)	Remarks
Toxascaris transfuga	Papaya seed suspension in Ringer Locke	1 50	72	Exposure to drug 18 hr Post exposure death time at 34°C in Ringer-Locke
	,,	1 100	98	,,
	,,	1 200	122	,,
	Piperazine citrate	1 50	6	,,
	,,	1 100	24	,,
	,,	1 200	60	,,
	,,	1 500	110	,,
	Control	0 100	236	,,
Ascaris lumbricoides var *suis*	Papaya seed powder suspension	1 50	61	,,
	,,	1 100	78	,,
	,,	1 200	114	,,
	Piperazine citrate	1 50	5	,,
	,,	1 100	22	,,
	,,	1 200	54	,,
	,,	1 500	98	,,
	Control	0 100	240	,,
Ascaris sp	Papaya seed suspension	1 50	97	,,
	,,	1 100	131	,,
	,,	1 200	192	,,
	Piperazine citrate	1 50	27	,,
	,,	1 100	103	,,
	,,	1 200	124	,,
	Control	0 100	384	,,
Pheretima sp	Papaya seed suspension	1 50	6	Death time in hr during exposure at 29°C in Ringer Locke medium
	,,	1 100	8	,,
	,,	1 200	12	,,
	,,	1 400	28	,,
	,,	1 800	42	,,
	Piperazine citrate	1 50	2	,,
	,,	1 100	3 5	,,
	,,	1 200	7 5	,,
	,,	1 500	14	,,
	Control	0 100	170	,,

Further experiments were carried out using earthworms as test organisms for examining the extractibility of the active principle by some solvents and also [illegible]

Results of the experiments on the solvent actions of distilled water, ammonium hydroxide, sodium bicarbonate, sodium hydroxide, hydrochloric acid solutions, ethyl alcohol, benzene, ether, carbon tetrachloride and Ringer-Locke

TABLE II

Extractibility of the vermicidal principle in papaya seed by various solvents

Test worm	Solvent	Vermicidal activity	
		Residue	Extract
Toxascaris transfuga	Distilled water	−	++++
Pheretima sp	,,	−	++++
,,	Dist water after 10 mins' boiling	−	−
,,	Normal saline	++	++
,,	Ringer-Locke	++	++
,,	Sodium bicarbonate (0 5%)	+++	+
,,	,, (1 0%)	++	++
,,	Sodium hydroxide (0 05%)	+++	+
,,	Ammonium hydroxide (5%)	+	+++
,,	Hydrochloric acid (1%)	−	−
,,	Ethyl alcohol	++++	−
,,	Acetone	++++	−
,,	Ether	++++	−
,,	Benzene	++++	−
,,	Chloroform	++++	−
,,	Carbon tetrachloride	++++	−

TABLE III

Stability of vermicidal principle in papaya seed on storage

	Storage condition Temp	R H (%)	Period (days)	Percent mortality on 5 hr exposure to papaya seed 5 mg/cc of Ringer-Locke
Seed powder	24°-29°C	68 75	0	100
,,	24° 29°C	68-75	15	0
,,	60°C		2	30
,,	26″ *Vacuum*		15	100
	24°-29°C		30	70
,,	*Air filled at atmospheric pressure*		15	70
	24°-29°C		30	50
,,	*Under* CO_2		15	80
	24°-29°C		30	80
,,	*Under Nitrogen*		15	90
	24°-29°C		30	70
,,	*Water extract freeze dried loosely stoppered*		15	60
	24°-29°C,	68 75	30	10
,,	*Freeze dried*		15	90
	water extract and stored		30	80
	under 26″ vacuum		180	70
Stored as seed	24°-29°C,	68 75	60	100
			180	100
	29°C	90	60	80
			180	50
Stored as powder	29°C	30	15	60
	29°C	90	15	0

R H = Relative Humidity.

medium on the papaya seed vermicidal principle are presented in Table II Extracts of papaya seed were prepared by shaking the powdered seed with solvents on a reciprocating mechanical shaker for 4 hr and the organic solvents were evaporated at room temperature under vacuum Alkaline and acid extracts were neutralized before use The extracts were tested for their vermicidal activities on the *Pheretima* sp The residues left after extraction of the powdered seeds with various solvents were also tested for their vermicidal potencies on their water extracts

Freeze-dried water extract of papaya seed powder was prepared and used in the experiment for its stability of the vermicidal activity on storage Results of the experiment on the stability of the active principle of the seed on storage under nitrogen carbon dioxide vacuum and air are given in Table III

Discussion

Results of the *in vitro* experiments revealed that papaya seeds contained a principle cidal to *Toxascaris transfuga* *Ascaris lumbricoides* var *suis* and *Pheretima* sp *Ascaris* sp from the polar bear was found to be relatively resistant to the action

the yield of extractive from papaya seed powder with normal saline solution water and Ringer's solution it was observed that higher yield was obtained with distilled water alone in comparison with water containing the solutes such as sodium chloride calcium chloride and potassium chloride This factor was probably responsible for the higher activity of the papaya seed in the test where water extract of the seed was used (*vide* Table II) However this requires further verification

Experiments on the solubility of the vermicidal principle of Papaya seed in

before extraction with the organic solvents Distilled water and dilute alkalies extracted the active principle from the papaya seed The residues left after extraction with distilled water and alkalies showed no or very little vermicidal activities (Table II) The extracts obtained from the water and alkalies after drying showed very high order of vermicidal actions which were indicated by the response of *Pheretima* sp in the test medium

Thermolability of the active principle was demonstrated in the experiment (Tables II and III) Air and moisture reduced the vermicidal activity of papaya seed powder on storage The whole seed maintained its vermicidal activity f r even six months without detectable loss at room temperature (24°C to 29°C) Papaya seed powder stored well under vacuum, nitrogen and CO_2

Acknowledgement

Authors are thankful to Dr V Subrahmanyan, the Director of the Institute for his keen interest in the work

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8 [illegible]
9 [illegible]
10 [illegible]
11 [illegible]
12 [illegible]

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STUDIES ON SOME OF THE EFFECTS OF *ASCARIS LUMBRICOIDES* IN VITRO AND IN VIVO

B K MALAVIYA AND C A GURSAHANI

From King George's Medical College Lucknow

Besides the mechanical obstructions brought about by *Ascaris lumbricoides* (round worm) in the various parts of the body which may bring about a mild derangement of the function of the organ wherein they may lie, or may bring about an acute surgical emergency these worms have been known to adversely affect the system of the host in many ways Their mere presence inside the body may make the host manifest a vast variety of allergic phenomena or may bring about disease in diverse ways

They are also known to elaborate anti tryptic substance for their survival in the host's intestines (1) This substance may interfere with the protein uptake [illegible]

getting ascaris did not grow as fast as the controls even though the diet was almost the same and contained all the vitamins salts and proximate constituents in balanced proportions further feeding experiments were undertaken In these later experiments higher amounts of ascaris were fed to the rats and in one group milk proteins were omitted

Rats were killed after sometime to see whether there was any macroscopical or microscopical changes in their viscera due to eating ascaris meat

Experiments were performed on the dogs to see if there was any general effect of the fresh human ascaris on blood pressure and respiration These effects were studied with total extracts filtered extracts and deproteinated extracts attempt was specially made to compute the quantity of worms which would cause deleterious effects

Ascaris were also tested for anti enzyme activity *in vitro* and finally, the rats were tested for allergy to see whether or not the adult ascarids whose normal [illegible]

For testing the anti enzyme act v t individual washed worms were ground in a porcelain pestle and mortar with cleaned and sterlized sand at 0°C and extracted in normal saline to give a dilution of 1 10 Anti trypsin, anti amylase and anti lipase activities were measured according to standard methods

Standard enzyme solution was taken to be the juice obtained from pancreatic fistulae made in dogs Enzymic activity of this juice was studied within 15 min. of its collection and was accepted as standard Any depressing effect on this activity *in vitro* at 37°C in one hour due to the ascaris extract, was studied and quantity inactivation of the pancreatic juice by one worm computed

It was expected that if the ascaris does bring about allergic reactions, feeding

he worm tissues
i kept for about
d by grinding a
(fresh weight)
red by grinding
vas either cloth
iated by using
made up to the

required dilution just before the experiment In all the cases, the extract was injected in the femoral vein of a dog and the dose in cc was the same as the weight of the dog in kg

Observation and Results

It was found that the weights increased equally in both the groups, *i e*, approximately 20 g per rat for the first 11 days when they were both fed with the control diet They increased equally for another 4 days after the introduction of the experimental diet and subsequently the increase was slower in the ascaris-fed group Thus on the 72nd day, the ascaris fed rats had gained 79 g on an average, whereas the controls had gained 97 g per rat

Ascaris meat was included in the diet of the experimental animals of a second group and the weight increase pattern was the same as in the earlier animals The ascaris fed rats gained on an average 23 g in 14 days, and 77 g in 64 days (53 days after commencement) whereas the controls gained 23 g and 96 g respectively

When 20% ascaris meat was included in the diet of the experimental animals the weight increase pattern was the same again as in the animals of group 1 and 4 The ascaris fed rats gained, on an average 23 g in 14 days, and 77 g in 64 days (53 days after commencement) whereas the controls gained 23 g and 96 g respectively

In a next batch of rats 20% of ascaris meat was fed but the proteins of the skimmed milk were replaced by wheat proteins

T'

very r

20%

only 1

or 68 g for the control group

As mentioned earlier the effects of extracts of ascaris were studied systematically The following Figs 1, 2 and 3 record the effects of total ascaris extract, filtered extract and deproteinated extracts

Those tracings show that there is a definite fall in blood pressure on injection of ascaris extract into the dogs, the pressure, however, returns to normal after a variable period if the dose is not too large The effective dose goes on increasing as the protein content of the extract decreases Thus 1/40 of the total extract caused a recoverable fall and 1/20 caused a fatal fall in blood pressure

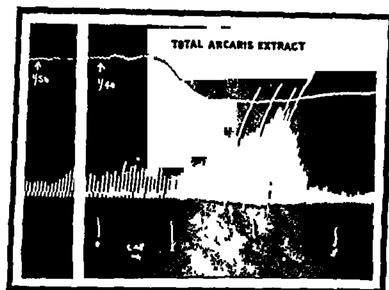


Fig. 1

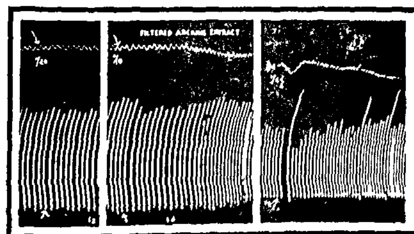


Fig. 2

With a filtered extract the fall [illegible] with the deproteinated extract [illegible] 1/1 was effective [illegible] and then also the effect was reversible.

There was almost no effect on resp[illegible]

Macroscopical and microscopical changes

There were [illegible] in the liver

TABLE I

Anti tryptic activity

Worm no	Weight (g)	Pancreatic juice inactivated (%)	Pancreatic juice inactivated per g (cc)	Pancreatic juice inactivated per worm (cc)
1	0.9	37.5	3.75	3.4
2	1.3	100.0	10.00	13.0
3	1.25	33.7	3.37	4.2
4	2.3	81.0	8.10	18.8
5	1.65	65.9	6.59	10.9
6	3.5	92.1	9.21	32.2
7	2.2	40.9	4.99	10.9
8	1.3	6.6	0.66	0.9
9	2.4	57.7	5.77	13.8
10	1.5	87.5	8.75	8.75
11	3.4	78.0	7.80	26.7
12	2.55	75.0	7.5	19.2
13	3.0	27.7	2.77	8.1
14	1.3	36.9	3.69	4.8
15	2.4	23.1	2.31	5.5
16	7.0	36.9	3.69	25.8
17	3.7	21.5	2.15	7.9
18	2.5	15.4	1.54	3.8
19	1.7	24.2	2.42	3.7
20	2.1	42.4	4.24	8.9
21	0.9	15.1	1.51	1.4

Average weight of the worm 2.33 g average percentage of inactivation 48.1, average volume of pancreatic juice inactivated by 1 g of ascaris 4.81 cc average volume of pancreatic juice inactivated by 1 worm 11.3 cc

1930 the appearance of parenchymal degeneration of the liver varying from ... and the ... intralobular veins in dogs, gui... *...hala* He ascribed these ... presence of such toxins ... ted in the adult worms used in our experiments It is possible that Blackie s results were due to allergens and not due to toxins It may be specially noted that the intestinal mucosa of the ascaris-fed animals was also normal

There is some substance present in the ascaris which lowers the blood pressure The effects are qualitatively same in all the 3 groups though, quantitatively, deproteinated extracts have only 1/20th effect of the total extracts These effects are qualitatively and quantitatively similar to those described by Emery and Herrick (6) and others They ascribed these changes to the presence of an albumose-peptone, named 'askaron' by Shimamura and Fujii (7) Similarly, Ino (8), reported the toxic effects of the body cavity fluid of the ascaris

These substances may be toxic but, they could not possibly have any effect clinically because the quantities required are comparatively large Thus, it would be necessary to inject deproteinated extract of 12 g (5 average size worms) of ascaris to cause troublesome shock in a 12 kg individual and a dose at least 10 times higher, *i e*, 50 worms, would be required to act *via* the intestinal mucus membrane, the spectacle of 50 dead worms being digested and absorbed instantaneously in the intestine of a 12 kg child is not ordinarily known to occur

T or 2 g of fresh worms per ra 240 worms for a child weigh nptoms Whatever be the generalized effects of ascaris, they are temporary and reversible and the animal can be restored to normalcy by removing the ascaris from his intestines

by the use of anthelmintics showing their causative role It is said that the presence of living ascarids in the human intestine can be diagnosed by performing various allergy, the rats can be sensitized to the ascaris tissue by 3 intra peritoneal injections and antigenic polysaccharides and lipids have been demonstrated in the ascaris In the present series the allergy experiments showed that the rats did not develop any sensitivity to the ascarid tissues even though they were eating such huge quantities of worms every day

In the absence of any definite local damage to the intestines or a generalized

infestations but it was eliminated in the present experiments as only the ascaris tissues were fed to the rats Hence, it is the latter mechanism through which the ascaris could possibly be retarding the growth of ascaris fed rats

The ascaris tissues do contain a trypsin inhibitor One g ascaris could inactivate trypsin co
the quantity o
correspond to
and to 1000 cc in the second It is therefore likely that tryptic activity of the pancreatic juice must have been interfered with inhibiting the digestion of proteins causing retarded growth

the pancreatic juice This reduces the tryptic activity of the pancreatic juice to a large extent and must, therefore, reduce the protein digestion very considerably bringing about insufficient availability of proteins and consequent retardation of growth It has been recently shown by Malaviya and Sindhe (17) that the tryptic activity of the duodenal juice of ankylostomiasis patients is only 63 1% of the normal Very obviously, *A lumbricoidis*, which have a greater mass than the ankylostomes will affect the tryptic activity still more and bring about considerable malnutrition

The retardation in the growth has been extremely pronounced in the rats fed on milk free diet where it amounts to 72% of the normal growth even though substitution of skimmed milk powder by wheat flour reduced the protein content by a bare 2% It is possible that protein content of the standard diet was marginal and just sufficient to keep the growth rate at a tolerable level In the presence of anti tryptic activity of the ascaris and a slight reduction in the protein intake has tipped the scales against the animals and the growth has come to a very low level

More probably, it is the quality of the protein which has produced these effects Shorb and Spindler (15) have reported that skimmed milk diet fed to young pigs infested with ascaris resulted in good weight gains since the host eliminated the parasites automatically Kiozami (16) also reported that pigs which were fed on milk and plenty of cod liver oil could not be experimentally infected with ascaris larvae It has been reported that malnutrition *per se* (even without intestinal parasites) reduces the tryptic properties of pancreatic juice presumably these properties would return to normal if malnutrition is corrected It would appear that milk proteins have some unexplained effects on the anti tryptic properties of ascaris which not only protect the host from the injurious effects of ascaris already present but actually help him to eliminate the parasites and regain proper nutriture at a still faster rate or even to resist the infect on gaining a foothold

SUMMARY

An attempt has been made to spot out anti nutritive or toxic factors in the body of adult *Ascaris lumbricoide* Protein free extracts of the worms had in high concentrations a mild reversible depressant effect on the dog s blood pressure

The growth of rats was retarded by includ ng fresh ascaris meat in their normal diet Exclusion of milk from the normal d et of these rats retarded weight gain further

Ascaris feeding for long periods did not have any sensitizing effect on the rats

Ascaris extracts were found to supress tryptic activity of the pancreatic juice in vitro

Except for a slight liver enlargement no significant changes were noted macrosopically or microscopically in the viscera of rats kept on high ascaris diet

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CONCLUDING REMARKS

DR B B BHATIA

At the outset, I would like to congratulate Dr Mukerji for having selected the topic of amoebiasis for this year's symposium which is of national importance to our country, and on his having collected workers of international fame in this field for discussion on its chemotherapy

That this disease has high incidence and is difficult to treat in the chronic form is evident from the fact that 50% of the patients who came to my consulting room complain of abdominal symptoms and 75% of them turn out to be suffering from chronic amoebiasis During the last two years 596 stool specimens were

Dr Srivastava in the city of Bombay

Dr Srivastava has reported incidence of 20% infection in healthy individuals From another paper, which you have just heard varied clinical forms of this disease have been discussed It is thus obvious that thorough research is necessary to determine the pathogenesis of the various clinical syndromes To what

weight, low blood pressure, some in which anxiety neurosis has developed during the course of this illness As regards the extra intestinal forms I have seen two cases of primary lung abscess, both of which were cured after emetine injections I have also *seen three cases of serious type of pleural effusion secondary to an* hepatitis, in which both the hepatitis and effusion were cured with emetine

As regards therapeutics I still consider emetine by far the most useful drug in the treatment of acute and extra intestinal form For the treatment of chronic amoebiasis, numerous drugs have flooded the market in recent years and an

to choose The superiority of each

onally I like to stick to old remedies

ful in giving relief to my patients

drug, my patients have come back

to me saying that my first prescription was better

I, therefore, feel that no outstanding advance has been made for the treatment of chronic stages In order to judge the clinical adequacy of new compounds, it would be most important to distinguish between relapses and reinfections, as long as patients are living in the same unhygienic environments chances of reinfections are there It would thus again be necessary to carry out therapeutic trials for long periods in special wards where chances of reinfections are obviated to see the effect of drug in producing permanent cures At present, my treatment of chronic cases consists of giving 5 days' course of camoform tablets (1 tablet four times a day) followed by one month's treatment with enterovioform (1 tablet

thrice a day) I very often give tablets of bellargol and yeast with enterovioform and with this line of treatment nearly all my cases are relieved Some of them, who went abroad to U K or U S A for a couple of years after this treatment have remained well, whereas many of those living in the old un hygienic environments came back with recurrence of symptoms after lapse of six months or a year However, we must keep our eyes wide open, and if some really superior drug is found for chronic cases it will prove a great boon to us

You have listened to some very interesting papers this morning, and I am sure everyone of you would like me to thank the speakers on your behalf and my own for the trouble they have taken in coming over from very long distances to take part in this very important symposium I also thank everyone of you for the co operation you have given, which has enabled me to run the papers according to the time schedule